

More rows about Europe's money

The British government is making a last-minute attempt to persuade the rest of Europe that it has a better way of creating a common currency. It has a good case, but one likely to be overtaken by events — and by the Deutschmark.

If people contemplating marriage prudently anticipate as many of the consequences as they can, why should countries planning union be more casual? Mr John Major, the British Chancellor of the Exchequer, could usefully have used that argument when, last week, he launched his last-ditch campaign against the European Communities' plan to legislate for a single European currency. Major says that the plan now proposed in Brussels is a recipe for enormous intra-European resentment. There is no doubt that he is correct; it is obtuse of national treasurers elsewhere in Europe not to recognize as much. This unsolicited expression of support for Major's isolated position is not biased by the circumstance that his own proposals for the future — the coexistence of national currencies with the European Currency Unit (ECU) — coincides with the interim solution advocated by this journal a year ago (*Nature* **341**, 89; 1989).

The correctness of Major's position is well illustrated by the monetary union of the two German states from the beginning of this month. People in the East will now spend a substantial part of their incomes on the products of factories in the West, with the result that many — estimates range from 10 per cent to 25 per cent, but reality could be worse — will be thrown out of work. That prospect is palatable only because the special circumstances promise that West German investment in the East will be so substantial and so quick that the economic upheaval will be short-lived. Let us hope so, for the sake of the civility of the united Germany.

The Brussels plan for European monetary union being pushed by the Commission president, M. Jacques Delors, would effect for the whole of the European Communities the kind of monetary union East and West Germany agreed last week, but without comparable compensatory arrangements. It is astonishing that the weaker economies in the European Communities, Greece and Ireland, for example, so casually applaud the Delors plan. Major is rightly worrying about Britain's future.

There may yet be a solution. The meeting of the heads of the European Communities' governments last week in Dublin convened not just one, but two, constitutional conferences in December on monetary and political issues respectively. They should be rolled together, for the second could allow the decisions of the first to be made acceptable. The model here is that of the United States, which enjoys the benefits of monetary and politi-

cal union within which 50 states retain a substantial degree of autonomy mistaken, in many a state capital, for sovereignty. In practice, the states raise funds from property and sales taxes and take responsibility for education, regional infrastructure and some aspects of welfare, while the federal government uses a nationwide income tax to pay for national defence and supplementary welfare costs. (The costs of federal research agencies such as the National Science Foundation and the National Institutes of Health are peanuts by comparison.)

In Europe, that arrangement is turned upside down. Defence and social security remain national responsibilities, financed by national income taxes, while the nearest thing to a central government (the European Commission) is financed by a sophisticated version of a sales tax, most of which is spent on keeping Europeans working as farmers when they should be doing something else. There is no mechanism by which economic imbalances will be offset by the flow of funds towards the poorer regions. (The European Communities have what is called a social fund, but it is too small to matter and is deliberately marginalized politically.)

Major's proper worry is that monetary union on the basis of fixed exchange rates (the ultimate Delors plan) will rob him and his successors of monetary freedom: interest rates will be determined by the market (and will be lowest in the most productive countries) while the device for repudiating debt called devaluation would be both illegal and impractical. (If West Virginia now sought to solve its economic problems by offering 90 cents for each federal dollar, it would quickly find itself in queer street.)

In the short run, Major is right; let national currencies and the ECU coexist while people get used to the idea, and so that the commercial banks that convert one European currency into another no longer enjoy a right to collect an arbitrary tax on every currency transaction. Further ahead, there can be no monetary union without preceding political union of some kind — at least an understanding of who pays for defence (and thus a common understanding of what defence is for) and a commitment by the rich towards the welfare of the poor. The sad truth about the argument about European monetary union is that there is as yet no serious constituency for either of these causes, and that December is not very far away. □



How did AIDS begin?

What is to be made of a death from pneumonia in 1959, after an apparent immunological collapse?

A THOROUGHLY compelling tale came to light in last week's issue of *The Lancet* — that of how it has been possible, by making use of the technique of the polymerase chain reaction (PCR) for amplifying pieces of DNA, to prove that a seaman who died at a Manchester (northwest England) hospital in 1959 most probably died of AIDS (G. Corbitt, A. S. Bailey & G. Williams, *Lancet* 336, 51; 1990).

There are two things to say, of which the most obvious is that the report is another vivid attestation of the power of PCR to identify particular nucleotide sequences in tissues long since dead in all clinical senses. On this occasion, by good fortune, some samples of tissue from the dead man had been preserved in blocks of paraffin wax. What now unambiguously emerges is that the kidney, bone marrow, spleen and pharyngeal mucosa (but not samples of liver and brain) carry nucleotide sequences characteristic of the virus believed to be responsible for AIDS, HIV (human immunodeficiency virus). Like Lazarus, it seems, the dead (and their secrets) have been brought to life.

The interest of this development is that it will throw further confusion on questions of the origin of AIDS, of its apparently rapid spread in the past decade and of its long-term potential. The year 1959 is a significant one because it is that from which dates the earliest sample of blood which is known to contain antibodies against HIV, collected in Zaire. So at least two adults died of what would now be called AIDS in 1959. It is unlikely that they were the only victims in that year. And if there were at least two victims in 1959, how many were there in 1958, and 1957 . . . ?

Nobody, of course, can know these things for certain. But the infection appears to have been much the same in 1959 as it is now. The dead Manchester seaman appears to have infected his wife and she the youngest of their three daughters, both of whom afterwards died of a similar syndrome. For what it is worth, the fifties was also the decade during which immunodeficiency diseases came to the fore under the general heading of 'leukaemia'. There was a ready explanation at the time: that previously, without the benefit of antibiotics, people with immune deficiency such as that associated with leukaemia had died of chance infection. It would be surprising if those deaths did not include a sprinkling of AIDS patients. That would agree with estimates of the time since the divergence of HIV from the simian immunodeficiency viruses from which they appear to be derived (see, for example, T. F. Smith *et al.* *Nature* 333, 573; 1988).

If there were sporadic AIDS cases during these years, why did the present spread of AIDS in developed countries not begin until the end of the 1970s? HIV is not

especially infectious, has a long incubation time, but eventually kills those infected. The sad case of the Manchester seaman shows how an isolated case of infection may run into the sand, with personal tragedy but no spread of infection beyond the immediate family. Given HIV, the rate at which the infection spreads is determined by the rate at which infected people acquire and infect new sexual partners in the interval between infection and death, with the transfusion of infected blood, the use of infected hypodermic needles and maternal transmission amplifying the process. All that is now reasonably well understood, as is the truth that the spread can be controlled only by individual precautions to ensure that sexual intercourse does not lead to infection by HIV and that other high-risk behaviour is avoided. The high incidence of HIV infection reported from many African countries is a sign of the end-point which, it is to be hoped, will elsewhere be avoided. □

Moving the goalposts

Disappointment with the World Cup could be simply prevented in future competitions.

NOT for the first time, the competition called the World Cup has ended with players and spectators alike complaining of their disappointment. One BBC commentator was heard to declare that "This hasn't been the best World Cup in the world". But the faults of these competitions, and of the game of football which is their substrate, are well recognized.

This time, the complaint is that too many matches ended with neither side having acquired a score, so that a "result" had to be manufactured, for the convenience of the organizers, by means of a humiliating game of chance in which people kick a football just beyond the reach of a person called a goalkeeper. But that is a superficial issue. It is much more important that the game of football, as at present played, invariably yields such small scores that they cannot possibly be an objective measure of the relative quality of two teams.

Plainly, the game needs radical redesign so as to increase the likelihood that scores different from zero will result. As things are, it is probably fair to represent teams' scoring potential by a Poisson distribution of the form $P(N) = \lambda^N e^{-\lambda} / N!$, where $P(N)$ is the probability of scoring N in a particular match, when λ will be the mean score against comparable opposition. A team's opponents will presumably have a similar distribution, but with a different parameter, say μ . A football game is or should be an experimental test of whether the parameters λ and μ are significantly different. Karl Pearson's F statistic is the appropriate test, but whatever is done, it is inconceivable that there could be a valid test of quality in less than, say, 12 hours play, which would clog all the television channels. It would be simpler to set the goalposts further apart. □

Commission to keep whaling moratorium

- Debate to be refought next year
- Value of scientific whaling disputed

London

THE International Whaling Commission (IWC) decided to keep the moratorium on commercial whaling in place for at least another year at its annual meeting in Nordwijk, the Netherlands, last week. Iceland and Japan failed to win approval from the commission for commercial quotas for minke whales but catches as part of 'scientific research' programmes will continue, despite the opposition of most IWC members.

Iceland had asked for a commercial quota of 200 minke whales in the central north Atlantic Ocean and Japan for 50 in the Okhotsk Sea. The minke is the smallest of the great whales and was the last to be hunted.

The Icelandic and Japanese requests had been expected as the moratorium, which came into effect in 1986, was set for revision this year. But the timetable for revision incorrectly assumed that the procedure by which catch quotas were set would be overhauled successfully by 1990.

All agree that a new system is necessary. Quotas depend on accurate population estimates, and information on whales' rate of reproduction. But these estimates are notoriously inaccurate, so that even catches within an agreed quota might drive a managed stock to extinction.

The IWC's scientific committee has produced a shortlist of procedures that may produce quotas that will protect stocks from overexploitation. But repeated computer simulations of their effects on whale populations are still needed.

The three main whaling nations — Japan, Iceland and Norway — accept that a comprehensive review of the commercial moratorium will have to wait until the 1991 IWC meeting in Reykjavik, Iceland. But Lars Walløe, scientific adviser to the Norwegian delegation, believes the anti-whaling nations will delay the adoption of a revised quota system there.

Irrespective of any political pressure, Phil Hammond, a UK delegate to the scientific committee, doubts if a new quota system will be in place in time for Reykjavik. Scientists are uncertain of the geographical boundaries between genetically distinct stocks of minke whales, and have to develop a quota system that will not threaten individual stocks, irrespective of where the boundaries lie.

Even if the new quota system is ready by next year, the anti-whaling majority of IWC members is likely to vote to maintain the moratorium. The whaling nations may

then carry out their long-standing threat to leave the IWC.

The controversial issue of whaling carried out in the name of scientific research is a more immediate concern. The scientific committee, meeting before the full IWC meeting, as usual disagreed over the merit of this year's research proposals. Like the full IWC, the committee is influenced by political considerations, with scientists representing the interests of whaling nations clashing with a committed core of anti-whaling biologists.

Debate over a Norwegian proposal to take five minke whales in the Barents Sea (to add to the 46 taken in the last two years) centred around the 'multi-species model' that Norway says will help the sustainable management of Barents Sea whaling and fisheries. Norwegian biologists plan to combine data on minke whale digestion and energetics with similar data for other species, to model feeding interactions.

But some members of the scientific committee doubt if the whale data, with such small sample sizes, will be useful. Sidney Holt, scientific adviser to the Seychelles delegation, argued that the Norwegians should first develop their model, and examine how the whale data will affect it, before taking more whales.

The much larger and ongoing Japanese programme on Antarctic minke (see *Nature* 344, 189; 15 March 1990) was the source of more disagreement. As in the past two years, Japan plans to take 300 minke whales, to estimate the age structure of the population.

Doug Butterworth, from the University of Cape Town, a scientific committee member not attached to any national delegation, presented analyses indicating that the Japanese data may be useful, allowing future quotas to be set with greater accuracy. But again, some biologists argued that the sample sizes are too small to estimate age structure. The committee needs a convincing analysis of the margin of error surrounding the age structure estimates, but is not satisfied that the Japanese have provided this. Although a catch of 300 minke will not seriously deplete the overall Antarctic population (estimated at about 750,000), some biologists believe that the analysis should precede further catches.

Fukuzo Nagasaki, scientific adviser to the Japanese delegation, agrees that a sample of 300 minke is "rather small", but says accurate age structure data will be

Reduction request

London

THE IWC, concerned about the huge catch of dolphins and porpoises in Japanese waters, has asked Japan to reduce its catch of Dall's porpoise to below 10,000 animals a year — the number taken in 1986. A report from the British pressure group, the Environmental Investigation Agency, published last month, estimated that at least 50,000 dolphins and porpoises are now killed each year off the Japanese coast.

Since the moratorium on commercial great whale catches came into effect in 1986, the shortage of whalemeat for the Japanese market has made porpoise meat a precious commodity, increasing the Dall's porpoise catch to about 40,000 per year in 1988. Although the 1989 catch was somewhat reduced, at around 30,000 animals, this figure still "must be unsustainable", according to one participant in last week's meeting. The entire population of Dall's porpoise in Japanese waters was estimated at only about 100,000 individuals in 1986.

But the resolution, proposed by a number of anti-whaling countries including the United Kingdom, can at most be described only as a forceful request. Fukuzo Nagasaki, scientific adviser to the Japanese delegation, acknowledges that the Dall's porpoise is a genuine concern. But he offers little hope that the new resolution will be obeyed. Although the Japanese government is trying to reduce catches, Nagasaki says, the fishery operates through a system of licences for boats, rather than fixed quotas.

A quota system would take "a long time" to implement, he says.

The Nordwijk meeting also passed a resolution asking IWC's scientific committee to gather information on the size of small cetacean stocks around the world, with the aim of presenting a report to the UN conference of environment and development in 1992. Peter Aldhous

collected in five or six years.

The full IWC meeting asked both Norwegian and Japanese governments to "reconsider" their permits for scientific whaling. Nagasaki says that some minor amendments to the research are possible, but the catch will go ahead. The IWC's constitution allows members to take scientific catches, irrespective of IWC resolutions.

The anti-scientific whaling lobby did gain a small victory, however, when a Soviet proposal to take up to 70 minke and 30 fin whales in the Okhotsk Sea was withdrawn. The Soviet plan was received poorly by the scientific committee, as the aims and methods of the research were unclear, and no Soviet scientists attended to explain the research. Peter Aldhous

Then there were four

Washington

THE long war over the fate of the 'Silver Spring' monkeys entered a new phase last week when three of the remaining seven monkeys kept at the Delta Regional Primate Research Center in Louisiana were killed after a series of experiments were carried out on their brain activity. As in the past, neither side in the fight over the fate of the monkeys was willing to soften their rhetoric. Both the animal rights groups and the congressmen who regard the monkeys as symbols of laboratory mistreatment of animals, and their opponents in the research community and the National Institutes of Health (NIH) continued to denounce each other's motives.

The office of Congressman Robert Roe, which tried unsuccessfully to have experiments delayed until this week in order for Roe to discuss the monkey's fate with William Raub, the acting head of NIH, was scathing in its criticisms. Gregory Simon, staff director in Roe's office, described NIH's decision to kill the monkeys as "a vulgar display of muscle", which appeared to be designed "merely to make the point that the scientific community is totally in control of animal research and will not let anybody else influence the way it is done". Roe's office threatened public hearings on NIH's conduct and renewed efforts to pass a bill that would force NIH to send the remaining monkeys to an animal sanctuary.

People for the Ethical Treatment of Animals (PETA), the animal rights group which has made the monkeys into a public issue, erected a protest sculpture, depicting the 'torture' of a monkey on a laboratory apparatus, outside the Department of Health and Human Services. PETA reminded the press that the monkeys are still the biggest cause of public appeals to the White House, claiming that it had received 45,000 letters last month asking Barbara Bush, the wife of the president, to free them.

But NIH continues to point out that it has acted correctly and according to the demands of the law. Raub said that it had sought permission to kill the three animals after their health deteriorated and veterinary experts had recommended euthanasia. "The department has made its intentions known for a long period," says Raub, "once the court matters were cleared up it was our obligation to proceed. A delay for an arbitrary period of time would be totally contrary to the representation we made to the court, Congress and everyone else."

Animal rights groups and NIH have agreed on virtually nothing from the start of the Silver Spring saga. According to PETA, when police recovered 17 of the

crab-eating macaques from the Silver Spring laboratory of Edward Taub in 1981, they found evidence that the animals had been mistreated. Taub, they claim, was later convicted of cruelty to animals in a Maryland court. Supporters of Taub disagree, pointing out that Taub was cleared of all charges on appeal and further exonerated in independent investigations.

The latest twist in the saga came after the Physicians Committee for Responsible Medicine, another animal welfare group, failed after a long period of court action to obtain an order to block the euthanasia of three of the monkeys.

Alex Pacheco, spokesman for PETA, says that for "NIH to paint this as a mercy killing is just another lie". He believes that the monkeys were used in experiments and then killed so that NIH could justify having refused for years to release the animals to a sanctuary. PETA claims that the experiments are bogus and points out that in 1985, Raub, who was then Deputy Director of Extramural Research at NIH, wrote that "we have no research protocols, ongoing or planned, for which these animals are appropriate".

Raub says that "it has been explained many times over that the letter was a response to a very narrow proposal". Since then times have changed and the monkeys have emerged as an extraordinarily valuable resource, not for the experiments on rehabilitation from injury which were originally planned, but to test new hypotheses concerning the degree to which the brain is able to reorganize itself after inputs to specific regions are lost. The Silver Spring monkeys had had nerves cut in their forearms.

Experiments conducted on the three monkeys before they were killed revealed "massive reorganization" in the cortex, according to Timothy Pons of the National Institute of Mental Health who lead the team. The data seem to confirm those gained from experiments carried out on one of the monkeys which was killed earlier this year (see *Nature* 343, 581; 15 February 1990). With data from four monkeys in hand, Pons says he will prepare a scientific paper for publication.

The significance of the science is not really the problem bothering Roe, who is chairman of the House of Representatives Committee on Science, Space and Technology, so much as the way in which the sensitive issue of the monkeys has been handled. Roe's staff members have spent the last six months, according to Simon, "working with PETA and with NIH to come up with the middle ground — an independent panel that would review the disposition of the monkeys". When agreement had apparently been reached, Simon

claims that NIH changed its mind. "Once they won in court everything changed and we're talking strictly 'might is right'", says Simon.

But Raub says that it was incorrect to say that NIH backed out of the negotiation. He said he had found it very "disheartening how little progress we made and how far apart the views of the animal rights groups and the NIH were. As we were making little progress there and the court restraints were being resolved a hundred per cent in our favour, and these animals had been judged by experts as candidates for euthanasia as far back as 1988, our obligation under the Animal Welfare Act was that we had to proceed with [the euthanasia] of those three animals".

Alun Anderson

AUSTRALIAN SPACEPORT

Soviet rockets gain US go ahead

Washington

PROSPECTS for the Australian Space Base at Cape York in northern Queensland are once more looking bright after US President George Bush indicated last week that he would not prevent US commercial satellites from being launched on Soviet-built rockets.

A major problem for the base has been that its likely customers were US companies wanting to launch US-made satellites while its cheapest source of launch vehicles lay in the Soviet Union. Until last week, the United States had objected to Soviet launches for US satellites, partly because they feared 'unfair' competition for domestic launches from state-subsidized Soviet organizations and partly because they feared that sensitive satellite technology might get into the hands of Soviet technicians.

The Cape York base, which will lie conveniently close to the equator, will cost around A\$350 million to build and could be ready for its first launches in 1995. The project is entirely commercial and is backed by a large Australian property developer, but the contract for running the base seems likely to go to a US company or to British Aerospace. It is not yet certain that the spaceport will be built, as environmental impact studies have yet to be completed and objections from aboriginal groups considered (see *Nature* 343, 400; 1 February 1990).

Tougher competition for satellite launches is bound to follow the entry of Soviet vehicles into the field, alongside Arianespace which is already well established, and the several US companies that are just beginning to provide commercial launches. A controversial US decision made last year also gave limited rights to the Chinese to launch US satellites.

Alun Anderson

AIDS POLICY

Slanging match with Sullivan

Washington

RELATIONS between the US government and AIDS activists took a turn for the worse last week when Health and Human Services Secretary Louis Sullivan released a statement calling on health officials to avoid all but "necessary and productive" dealings with the radical protest group ACT-UP (AIDS Coalition to Unleash Power).

The statement is Sullivan's response to a noisy demonstration, led by ACT-UP, which drowned out his closing speech at last month's International Conference on AIDS in San Francisco. Upset by the attack, during which he was pelted with wads of paper and other makeshift missiles, Sullivan now says that he will not "let [his] department be used as a punching bag" or "have dealings with people who behave as ACT-UP did".

Peter Staley, a spokesman for ACT-UP, says that initially his organization took Sullivan's statement "very seriously". If the threats implied by this "politically naive" statement were carried out, says Staley, "it would be tantamount to a declaration of war".

But the Secretary's bark may well prove bigger than his bite.

When pressed for details, James Brown,

a spokesman for the Public Health Service, was unable to give specific examples of the kinds of dealings with ACT-UP that will be curbed, although he said that the positions currently held by members of the group on research advisory panels are not in jeopardy. The Secretary will not stop "certain programmes" from drawing on ACT-UP's knowledge and expertise, Brown said.

ACT-UP says that so far there has been no evidence of any pressure on their many contacts in the Food and Drug Administration and National Institutes of Health (NIH) to stop communicating with its members.

Sullivan's words come at a time when activists and public health officials, particularly at the Food and Drug Administration and NIH, are nurturing a growing relationship. Members of ACT-UP currently sit on two of NIH's research advisory panels and expect to be awarded more committee places when the AIDS Clinical Trial Group at the National Institute of Allergy and Infectious Disease, which oversees the bulk of US AIDS research, is opened up to community representatives later in the year. "We certainly hope those won't be jeopardized", says Staley.

David Concar

AIDS EPIDEMIOLOGY

Another early case identified

Washington

THE date of the first known death from AIDS has been pushed back to the 1950s. Researchers at the medical school of Manchester University, England, have found evidence of HIV (human immunodeficiency virus) genetic material in tissue specimens from a 25-year-old caucasian seaman who died of severe pneumonia in Manchester in 1959.

Previously, the earliest recorded cases of HIV infection, identified retrospectively, were in three members of a Norwegian family who contracted AIDS in the 1960s and died in 1976. HIV antibodies have also been found in a blood sample collected from a patient in Zaire in 1959 — but in that case there is no evidence that the patient died from AIDS.

The new finding will not initiate a rethink in the epidemiology of HIV infection. According to Roy Anderson of Imperial College in London, epidemiological analysis of high present-day infection rates in places such as Malawi indicates that HIV must have been present in Africa for at least 30 to 40 years. More reports of early sporadic cases of AIDS will emerge, says Anderson, as researchers look back over the records. He also emphasizes that the Manchester seaman represents an unlikely

point of origin for the current AIDS epidemic in Britain.

To obtain evidence for HIV infection, the Manchester researchers used the polymerase chain reaction (PCR), a technique that allows trace amounts of genetic material — in this case proviral DNA — to be amplified so that its identity can be determined using a DNA probe. One drawback with PCR is its sensitivity to contaminants, a problem which in the past has led to false results. Gerald Corbitt, one of the three researchers who reported the case in the British medical journal *The Lancet*, says that every precaution was taken to prevent contamination of tissue samples. They performed the analysis in a "remote" laboratory and their PCR assay gave the same result when repeated on different tissue samples.

The researchers do not yet know whether the patient was infected with HIV-1 or HIV-2, but are planning to sequence the proviral DNA to find out. Their main limitation is a dwindling supply of genetic material.

Sadly, there is little information on the seaman himself — his earlier medical history and the countries he visited before 1959 are apparently unknown.

David Concar

BIOLOGICAL WEAPONS

Lawyer talks tough

Boston

IN the coming months, many biomedical researchers are likely to receive a disturbing memorandum in their mail, warning them that their research could be breaking the law and they could be punished by imprisonment for life. The memorandum will come from the office of Francis A. Boyle, a professor of international law at the University of Illinois at Urbana-Champaign who was instrumental in drafting recently passed legislation which incorporated provisions of the international Biological Weapons Convention into US domestic law (see *Nature* 345, 192; 17 May 1990).

Boyle's memorandum, addressed to "all life science researchers" and circulated first to several scientific publications including *Nature*, alerts researchers to the provisions of the Biological Weapons Anti-Terrorism Act of 1989, Public Law 101-298. In effect, the law makes it a federal crime for a US citizen "knowingly" to develop, produce, stockpile, transfer, acquire, retain or possess "any biological agent, toxin, or delivery system for use as a weapon", or to aid a foreign state or any organization to do so.

Chuck Dasey, spokesman for the Army's Medical Research and Development Command, says that the Army has abided by the Biological Weapons Convention since its inception and thus by definition abides by the new legislation as well.

In a telephone interview last week, however, Boyle stated that the Army's contention is precisely what may now be challenged through either civil or criminal lawsuits involving specific research projects. In his memorandum to researchers, Boyle states that in his "professional opinion" many research projects that have already been funded by the US Department of Defense's Biological Defense Research Program (BDRP) "raise serious compliance problems" with the new law. Consequently, Boyle urges researchers who are receiving or applying for funding from the BDRP to "obtain legal advice and counsel from a competent attorney" about their research or grant application.

Boyle is not shy about his opposition to the BDRP programme. He stresses that in his view, "the first step is to warn researchers about the law" in the hope that they will look carefully at whether their work is fully in compliance. For researchers who continue to receive military funding, however, Boyle acknowledges that he may be personally involved in a legal challenge against them in the future if their work appears to him to stand in violation of the law.

Seth Shulman

And now some good news

Paris

AFTER 11 months of calculation and rethinking, scientists at the European Space Agency (ESA) now believe that the seemingly ill-fated astronomy satellite, Hipparcos, will be able to perform its mission successfully after all. A request for ESA to build a follow-up, Hipparcos 2, has been dropped.

Hipparcos was launched last August by an Ariane rocket with the intention of gathering information on the position, proper motion and distance of 120,000 stars to a new order of precision. The launch itself was perfect — but then the apogee boost-motor failed, leaving the satellite in a highly elliptical orbit, rather than the geostationary orbit that was originally planned (see *Nature* 340, 581; 1989). The load thus put on the satellite's three solar arrays seemed likely to limit the mission to a duration of six months, rather than the planned four years. Data collection also became a problem as the elliptical orbit put the satellite out of reach of a single tracking station and transmissions were regularly obscured as the satellite passed through the Van Allen belts.

Since then, help to track Hipparcos has been found at ground stations in Perth (Australia), Kourou (French Guiana) and Goldstone (United States), adding to the planned activities at Odenwald in West Germany. That means that the satellite is visible 93 per cent of the time and useful observations can be made 60 to 70 per cent of the time.

The future of the mission depends on the rate at which voltages from the solar panels decline. Mike Perryman, Hipparcos project scientist at ESA, last week said that projections based on rates collected over several months suggest Hipparcos may last two to three years "or four or even longer". That will allow the 120,000 stars of the input catalogue to be positioned with 10–20 milliarc accuracy, still, according to Perryman, 10–20 times better than could be achieved from the ground.

For Perryman, the effect of the failure is now on the quantity of observations, not their quality. "We cannot retrieve quite as much data, but for the 70 per cent of the time when we do get data, the quality is outstanding. We are delighted." Over 5 million observations have already been made of the 120,000 stars.

An 11-man enquiry board set up to investigate the failure of Hipparcos submitted its final report and recommendations last week. French defence department missile expert Daniel Reydellet, who chaired the inquiry board, said in Paris that an 'off-the-shelf' component in the ignition circuit of the apogee booster

motor — the through bulkhead initiator (TBI) — apparently did not work. But Reydellet was reluctant to say whether the TBI, made by the California-based company, Hi Shear, was faulty. The TBI depends for correct functioning on pulses from other parts of the ignition circuitry.

The report nevertheless suggests that Hi Shear's reputation with the US National Aeronautics and Space Administration may have dazzled ESA engineers into foregoing quality control checks. When, as part of the inquiry, experts visited Hi Shear, Reynellet said they found that

EUROPEAN SPACE PROGRAMME

Columbus shipwrecked?

Munich

A SMOULDERING dispute over the West German space programme flared into life again last week when the 1991 budget of the West German Research Ministry (BMFT) was announced. Space research and technology accounted for half of the 4 per cent increase in the budget, prompting renewed criticism from the opposition parties that BMFT was taking money away from other areas by investing so much in manned space flight.

With the estimated cost of the European space station Columbus, which is supposed to complement the US space station Freedom, having risen by thousands of millions of dollars, West Germany has found its role as main developer of Columbus, bearing 38 per cent of the cost, increasingly difficult to shoulder. When the European Space Agency (ESA) council decided in 1987 to begin three linked space projects — Columbus, the space shuttle Hermes and a heavy payload rocket Ariane-5 — the total cost was estimated at DM 21,000 million (\$12,800 million).

Since then, the price has risen to an estimated minimum of DM 30,000 million, according to opposition members of the West German parliament. BMFT refused to confirm any such figure, but admitted that large increases would be necessary in 1992 and beyond to cover the rising cost of the programme. West German Research Minister, Heinz Riesenhuber (Christian Democrat), has promised not to let space research take more than 22 per cent of BMFT's budget. That proportion is now 20 per cent.

Columbus is meant to be both a vehicle for basic research in microgravitation and a platform for industrial research and development, but critics claim that neither goal will be met by the current plan. Opposition Social Democratic parliamentarian Edelgard Bulmahn doubts that the latest US plan for space station Freedom

TBIs originating from the same batch as the one used in Hipparcos had "disappeared", thereby ruling out fresh tests. Because the components can be used only once, statistics from the testing of a whole batch are needed to assess reliability. Nevertheless, analyses being carried out by ESA have suggested that quantities of explosive may have been only just within the required specifications.

Wise after the event, the inquiry board has recommended that, when off-the-shelf components are bought in future, ESA engineers should monitor manufacture more closely. But, given the complexity of modern satellites, this may not be realistic.

Peter Coles

— to use it as a stopping-place for missions to the Moon and Mars — is compatible with using Columbus for microgravity research.

Anticipating a major budget battle, BMFT requested as early as 1987 that ESA cut its budget for the three projects by 15 to 20 per cent. But Buhlman says that cuts of this size would reduce the amount of new technology that European countries could develop, forcing ESA to buy much of the needed technology from the United States.

The members of Helmut Kohl's centre-right coalition gave a vote of confidence to Columbus last month before parliament adjourned for the summer. But the support may be conditional. Christian Democrat parliamentarian Christian Lenzer says he is "totally against building something that industry is not interested in using," sowing the seeds for a conflict with BMFT, which defends the project as an important investment in basic research. And a spokeswoman for the prime contractor for Columbus, MBB-ERNO in Bremen, said that "it will take 15 years" before the space station "becomes a business" the way that satellites or launch vehicles have.

Adding to the confusion, an ESA review board gave the MBB-ERNO proposal a low rating; the proposal was judged to be "of a generally low level of quality", and the "quality and accuracy of the cost data" was declared "totally unacceptable, with an extremely low level of credibility". An ESA spokesman said it was normal for a large proposal to receive a negative evaluation early in the approval process.

Next year, ESA member states will meet to decide how to proceed with the three space projects. If costs rise more than 20 per cent from original estimates, member states have the option to withdraw from the programme.

Steven Dickman

SPACE SHUTTLE

More trouble for high fliers

Washington

THE temporary disablement of two space shuttles by leaks in the hydrogen feeder lines between the external tank and the orbiter is almost certain to bring extra delays to one or more long-planned astronomy missions, adding to the pain caused by the revelations of manufacturing flaws in the Hubble Space Telescope (see *Nature* **346**, 3; 1990).

Astro-1, an ultraviolet and X-ray astronomy package (see *Nature* **345**, 375; 1990), is waiting for space shuttle Columbia to be repaired after a hydrogen leak was detected shortly before launch. The Gamma-Ray Observatory (GRO) is supposed to fly on shuttle Atlantis in early November, but Atlantis is now stranded on the launch pad at the Kennedy Space Center, Florida. In the meantime the joint US-European solar probe Ulysses must be launched by Discovery in October so that Jupiter is in the right place to give it a gravity-assist into a polar orbit around the Sun. Even if Columbia or Atlantis were fixed in the next few weeks, the chances are high that Astro-1 or GRO would have to be postponed to let Ulysses fly on time.

The leak in Atlantis was discovered after NASA (National Aeronautics and Space Administration) mission controllers decided, in the light of their experience with Columbia, to conduct a pre-launch 'tanking test' in which the external tank was filled with hydrogen as it would normally be just before launch. They detected a small hydrogen leak, and postponed the Atlantis flight while it and Columbia were investigated. By this time, the various 'umbilical' pipes connecting Columbia to its external tank had been taken apart and tested individually, but the leak could not be reproduced.

Last weekend, NASA engineers tested Columbia's reassembled umbilical system at the Rockwell Downey company, near Los Angeles, where they were built.

This week, Atlantis was being prepared for a new launch-pad test, in which various parts of the umbilical will be surrounded by sealed bags in order to determine which part of the assembly is leaking.

How the launch schedule will be put back together depends on the amount of work needed to fix the leaky sections. If repairs can be made on the launch pad, Atlantis and its secret payload would be sent off as soon as possible, which would probably delay the launch of Astro-1 until after Ulysses. If Atlantis has to be rolled back off the launch pad to be repaired, Columbia and Astro-1 could be launched instead, but then GRO could not be launched until Atlantis's Defense mission had been completed.

David Lindley

LAWRENCE LIVERMORE LABORATORY

Bargain hunters' paradise in California

Washington

LAWRENCE Livermore Laboratory may be one of the largest US nuclear weapons research and development laboratories, with a strong reputation for its 'Star Wars' research, but it sounds a lot more like the thieves' bazaar of old Baghdad in the just published report of an investigation by the General Accounting Office (GAO).

The report says that equipment worth more than \$45 million has vanished from the laboratory, which is managed by the University of California, including 49 microcomputers, 205 typewriters and word processors, 841 pieces of video equipment, 388 cameras and related equipment and thousands of calculators. Where has everything gone? A statement put out last week by the office of congressional strong man John Dingell, chairman of the House of Representatives Energy and Commerce Committee and its oversight and investigations subcommittee, noted that allegations had been made at a congressional hearing that "laboratory equipment, such as computers, was stolen and traded for drugs". Dingell, who ordered the GAO

investigation, claims that at the laboratory "[w]e have seen drug use, cover-ups, retaliation against concerned employees, poor safety and poor security".

The Department of Energy (DoE) disagrees. In a reply, DoE diplomatically thanks Dingell for his interest, saying in a statement from its San Francisco office that it "consider[s] the findings helpful to improve property management at the lab" and that "improvements are under way". But it also says the laboratory has already located 98 per cent of the 'missing' property. According to DoE spokeswoman Caroline Powell, the San Francisco office has checked that the property is really in place. She says of the GAO failure to find many items, "maybe the thing was in a desk drawer and they didn't ask the person who was responsible".

GAO has yet to report on a separate investigation into whether any nuclear materials or classified documents are missing from the laboratory. Dingell has promised to hold hearings later this year to examine the report and DoE's claims.

Alun Anderson

EUROPEAN SCIENCE

New Framework row

London

PLANS by European Community (EC) research commissioner, Filippo Pandolfi, to concentrate EC basic science spending almost entirely on postdoctoral fellowships have set him at odds with member states' representatives on CREST, the EC's scientific and technical research advisory committee. The disagreement could delay spending on basic science through the Framework programme, which supports all research and development efforts in the EC.

EC funds for basic science are set to increase under the third Framework programme, which will run from 1990 to 1994. The increase itself has caused apprehension in some members of the UK research councils, who fear that UK spending through the EC will be taken from the domestic British science budget (see *Nature* **345**, 376; 31 May 1990). But Pandolfi's decision to initiate a 'human capital and mobility' programme which would support several thousand postdoctoral positions has attracted wider opposition.

Only Italy, the present incumbent of the EC presidency and anxious to avoid rifts, has so far not come out against Pandolfi's plan.

Pandolfi argues that promoting the exchange of young scientists between EC member states is useful because it is not strongly supported by individual states. But CREST is concerned that there

simply will not be enough young scientists to fill the places, and in any case wants a more diverse programme, with support for postgraduate scientists and large research institutes.

Although the proposed 518 million ECU (1 ECU = £0.70) human capital and mobility programme may include some support for applied science, it is envisaged that it will largely take over from Framework's SCIENCE programme, which is now the centrepiece of EC basic research support.

Supporters of the present diversified system point to the continued success of the SCIENCE programme's research projects, particularly the huge collaborative project on NdFeB alloy-based magnets (see *Nature* **338**, 734; 1989), which has given Europe the lead in this area. These large-scale projects do, in any case, support a number of postdoctoral researchers.

The current SCIENCE programme is due to end in 1992, but Pandolfi says he plans to fund a continued programme up to 1994. This will depend on additional money becoming available, however. Sir Peter Swinnerton-Dyer, chairman of the committee that allocates funds under the existing SCIENCE programme, is concerned that there could be a delay in getting the new programme under way, if CREST and Pandolfi cannot quickly reach a compromise.

Peter Aldhous

Not in my back yard

Munich

As the barriers between Eastern and Western Europe come down, some nuclear power plant operators in the West are wondering if they may not now have a golden opportunity to set up some of their unpopular generating facilities next door. The Czechoslovak government has already begun negotiations with French and German companies to build plants at the Temelin site, near the Austrian border. The plants would be financed by selling electric power to Italy, transmitted via high-tension cables running through Austria.

The idea of building new nuclear plants in Czechoslovakia is controversial, especially in neighbouring Austria, which abandoned its own nuclear programme in 1987. Anti-nuclear activists based in Austria have been campaigning for years against existing Czechoslovak reactors.

Nevertheless, there are clear benefits for Czechoslovakia in expanding its nuclear programme in this way. It could gain access to important Western technology and at the same time reduce its dependence on high-sulphur coal, which has devastated the environment (see *Nature* 344, 91; 1990).

Czechoslovakia currently derives 27 per cent of its electricity from nine Soviet-built nuclear reactors at three sites. Another seven reactors that are under construction will raise dependence on nuclear power to 45 per cent by the year 2000. The design of two reactors, at Bohunice near the Austrian border, has been criticized as fundamentally flawed. They are of the same type (VVER 440-V 230) as those at Greifswald, East Germany, which were last month judged to be hopelessly unsafe by a West German safety commission and ordered to be shut down. There are four reactors of the same type still operating in Bulgaria and four more in the Soviet Union (see *Nature* 344, 282; 1990). The remaining seven Czechoslovak 440 MW reactors are of a slightly more modern design.

So far, the newly elected democratic government of Czechoslovakia has continued its Communist predecessor's support for nuclear power. As recently as 27 June, an adviser to the now-defunct Ministry for Fuels and Energy, Frantisek Suranski, declared on Austrian television that Czechoslovakia "sees no reason or option to give up nuclear power now". Even the activist Environment Minister for the Czech republic, Bedrich Moldan, has said that he favours the continued operation of existing nuclear plants and the completion of plants under construction.

Prime Minister Marian Calfa announced on 3 July that several coal-burning plants

in northern Bohemia would be closed by the end of the year. That decision will put more pressure on nuclear plants to fill the resulting energy gap. A moratorium declared in January by the interim government on construction of the two newest 1,000 MW plants at Temelin does not mean that nuclear power is out of favour — these are the plants that the government may replace with new Western designs.

Austrian objectors to nuclear power have made several arguments against Czechoslovakia's plans to expand its nuclear capacity. They point out that no one has studied the real cost of Czechoslovak energy or how much Czechoslovak industry could improve its energy-efficiency. According to Michael Undorf of the Austrian Greenpeace organization, the Austrian government has offered to form a joint commission to study this problem, and Moldan has tried to get the Czech government to accept, but without result.

Once Czechoslovakia carries out such studies, said Vienna University ecology professor Bernd Lötsch on Austrian television on 27 June, the Czechoslovak government will realize how much more effective it is to improve the efficiency and environmental standards of industry and existing power plants than to build new nuclear plants. Undorf agrees, saying "We hope that [the prohibitive cost of new plants] is what ultimately defeats the idea."

NUCLEAR CLEAN-UP

Runaway costs cause sharp reduction

Boston

ESTIMATES of the cost of the first phase of the immense project to redress environmental damage occurring at US government facilities used to manufacture nuclear weapons have doubled in less than a year, according to projections released last week by the Department of Energy (DoE). The first five years of the 30-year clean-up are now calculated to require more than \$28,000 million. The entire clean-up operation is expected to cost in the vicinity of \$100,000–\$200,000 million dollars over the next few decades.

DoE says that the projected costs are rising because of a better assessment of the job's technical requirements, and because of the increases in charges that are demanded by contractors to protect themselves against the lawsuits that may arise during the clean-up procedures. Provisions in current hazardous waste laws, as well as increases in environmental litigation by the states and the Environmental Protection Agency, conspire to make contractors increasingly wary, according to Leo Duffy,

But conservation alone will not solve the Czechoslovak energy problem, according to Thomas Horacek, a researcher in an institute attached to the former Czechoslovak Energy Ministry. Czechoslovakia is a net importer of electricity from other Eastern European states, and more electricity will be needed relative to other forms of energy as the country modernizes its economy, he says.

Michael Schneider of the Paris-based World Information Service on Energy points out that schemes to finance new nuclear plants by exporting electricity have never worked in the past. Western nuclear power plant producers came close to realizing a deal with Turkey between 1983 and 1987. In exchange for a complete plant built by a Western company, Turkey was to have exported power for 15 years. But by 1987, both West German and Canadian companies had pulled out of the scheme.

If Italy does not in the end prove a willing customer, it is difficult to imagine there being any other market for electricity in another nearby country. Before German unification began, both West Germany and France were producing electricity surpluses, and by the time Czechoslovakia is in a position to build new plants, West German utilities are likely to have met the entire demand in what is now East Germany. Environmentalists have already vowed to fight if Austria moves towards approving the construction of power lines to Italy from Eastern Europe.

Steven Dickman

director of DoE's environmental management office.

Responses to the new cost estimates were mixed. While the Energy Department continues to win high marks for the *glasnost* fostered under Energy Secretary James Watkins' watch, critics complain that the department is absorbing the costs of cleaning-up into operating budget as a way to maintain personnel numbers even while facilities are being shut down (see *Nature* 342, 5; 1989). Some observers think that clean-up costs are likely to go higher still, so high that it may not be politically feasible to pay them.

Even if Congress does allocate the necessary funding, the current cost projections and the operation itself are predicated on the assumption that the Department of Energy will successfully complete its nuclear waste repositories in Nevada and New Mexico. But both of these facilities are far from completion and continue to be fraught with political opposition and technical problems.

Seth Shulman

Imaginary orderliness

SIR—The situation at the Natural History Museum (NHM) is as serious as any that has faced Britain's 'national' museums in recent times. Yet the bulk of Sir Walter Bodmer's Commentary (*Nature* 345, 569; 1990) appears to ignore reality in a push for the status quo.

Professor John Evans, immediate past president of the Geologists Association, through his comments (reported by Henry Gee in *Nature* 345, 99; 1990) on the corporate management proposals of the museum's director, Neil Chalmers, is working to advantage only the agenda of those intent on pursuing the narrow rational economic agenda. Statements such as "[the plan] has . . . been cobbled together in American-style business jargon presumably courtesy of Disneyland where many of the museum's administration went to learn such jargon" can surely be described only as ignorant nonsense.

The NHM is studying Disneyland so as to give visitors better service: that is good. Disneyland is expert at excellent customer services. That contributes more to the continuation of the NHM's outstanding work than abuse from single-interest groups who wish to brand everything about management as a fascist plot.

Evans' successor, Professor Beverly Halstead, long-time critic of changes at the museum in exhibitions which are based on very good research and sound judgement, has also done little to help the museum by his recent comments.

Detailed analysis of the corporate plan and of whether it is an appropriate response to the future is well worthwhile. But that exercise should surely use the same caution and logic as is applied by scientists to their own research.

The problem is the British government and its continued refusal to recognize the enormous importance of museums in all areas of science and education. And its failure to recognize their importance in economic terms is shown by recently published studies (for example, *The Economic Importance of the Arts* by John Myerscough, Policy Studies Institute, July 1988).

To help the museum and its staff and (most of all the public), the last thing one should do is to campaign on single-issue agendas such as taxonomy and the 'undesirability' of changing direction. I am astonished at the narrowness of view of many of the scientific groups expressing concern. Reading some of the draft letters that are circulating, one would imagine that the only problem resulting from reductions in the museum's funding was the loss of knowledge of sponges or crustaceans. Of course this is important, but it is the existence of the museum which is at risk. Only if that is secured will it be

possible to discuss its shape and future programmes.

What would the Geologists Association do to reverse the trend at the museum towards salaries exceeding the total money granted by government? Presumably 132 years have taught them something. Try joining together with others concerned about the future of museums to put pressure on the government. It is a political issue after all.

DES GRIFFIN
(Chairman, Council of Australian
Museum Directors)

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SIR—Sir Walter Bodmer, writing as chairman of the trustees of the Natural History Museum (NHM), has defended the recent corporate plan of this museum¹. He argues that, given a shortfall in funds, the scientific programme was the only area left in which cuts could be made. He points out that many scientific posts have been lost in the museum over the past five to seven years by random natural wastage and claims that, because of this randomness, "the director and his senior staff [have had to] assess research priorities" so that any further cuts would occur in a more orderly way.

Such orderliness is imaginary, however, for the imposed cuts do not fit any scientific plan. They were decided by heads of departments in obedience to the command: "Your department will lose x posts and you will decide which ones".

This command produced many anomalies where the redundancies run counter to the ostensible aims of the plan. To give one example out of many², the museum, under the plan, is supposed to be more environmentally aware than in the past — and we accept this aim. Nevertheless, research is to stop in diatoms, which are first-class environmental indicators of particular value in reconstructing the course of global warming. Many of these anomalies have been mentioned in parliament by Mr Tam Dalyell MP³.

The brutality of the plan, the high-handedness with which it was announced, and other insults dealt to us by management, have demoralized the staff and produced many requests for voluntary retirement additional to those which the management originally called for. It is true that these requests are randomly distributed scientifically, but so are the cuts proposed by the plan. Allowing some requested retirements would provide several free posts and enough money to restore some of the axed curatorial and research positions. We therefore urge

management to reassess the cuts with all this in mind.

After all, Sir Walter Bodmer would never claim that the museum is stronger without several axed scientists who dearly wish to stay, including a certain world-famous archaeozoologist and an internationally known palaeobotanist.

P. E. J. WHEATCROFT
(Branch Chair)
R. P. S. JEFFERIES
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1. Bodmer, W. *Nature* 345, 569–570 (1990).
2. Science Defence Committee *Corporately Planned Damage to Science* (IPMS (Natural History Museum Branch), 1990).
3. Dalyell, T. *Hansard* 174 (118, for 11 June 1990), pp.18, 77, 102.

Journal titles

SIR—I commend Dr Judah Rosner's comments on the unseemly dogmatism of the AST (assertive sentence title). Perhaps he is right in suggesting that some scientists embrace this style in order to impress granting agencies. But there is one other factor. In 1981, Martin Glennie and I submitted to *Nature* an article with the fuddy-duddy title "The use of univalent antibodies to kill tumour cells". It was published as the resounding "Univalent antibodies kill tumour cells *in vitro* and *in vivo*". Had we in the intervening months become more thrusting and positive? Not in the least. The title was altered in the editorial office of *Nature*.

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Eminent engineers

SIR—Not only was President Herbert Hoover an engineer (*Nature* 345, 106; 1990), but he had strongly held views on the value of education. He wrote in 1941 that "British universities [had] refused to incorporate engineering into their curricula until much later than the Americans. In the meantime, the American engineers — especially the mining engineers — flooded the British Empire" (Foreword to T. T. Read's *The Development of Mineral Industry Education in the United States*, New York, 1941).

We would certainly be helped if Hoover's positive attitude to training engineers prevailed in British politics today.

HUGH TORRENS
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Opportunities for the East

SIR—Further to the News article "Opportunities for the East" (*Nature* 345, 196; 1990), readers may be interested to know that the International Agency for Research on Cancer (WHO), based in Lyon, France, runs a fellowship programme for research training in cancer addressed to young postdoctoral scientists. This programme was initiated in 1966 and has already provided over 70 fellowships to Eastern European scientists, thus contributing to the maintenance of contact with the international scientific community and to the development of cancer research in the home country of the Fellows.

R. MONTESANO

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Hungarian freedom

SIR—Steven Dickman writes (*Nature* 344, 615; 1990) that "The old Hungarian University became a Romanian one in 1920, when Transylvania was given to the Romanians as part of the peace of Trianon. But the Romanian faculty was forced to flee to Sibiu in 1940 when the Axis powers gave the area back to Hungary". He omits to say that the original Hungarian faculty was forced to 'flee' to Szeged in 1920. In 1940, the original Hungarian University of the city (Kolozsvár in Hungarian, Cluj in Romanian) was permitted to come back. After the Second World War, according to the agreement overseen by the victorious four powers of Britain, France, the Soviet Union and the United States, it was stipulated that Romanian and Hungarian universities should coexist. "Until 1959 Romanian and Hungarian universities existed side by side, then they merged into a predominantly Romanian entity", according to Dickman. This is not exactly what has happened. The truth is that the Hungarian faculty was suppressed on orders from Bucharest. An eminent professor at the Hungarian university committed suicide and in his farewell letter wrote that his act was a protest against the violation of liberty and the decree of suppression.

When the university was founded in the last quarter of the nineteenth century, more than 80 per cent of the inhabitants of the city were Hungarian. This is evident from the tombstones in the city's one cemetery. More than 80 per cent of those buried before 1920 were Hungarian. Many great Hungarian writers and musicians (Bartók and Ligeti, for example) were born in Transylvania.

We should also remember that the principle of the Austrian Empire was "*Divide et impera*" ("Divide and rule"). The Austrian emperor always listened favourably to the different ethnic groups of the empire.

Dickman states that there are now "30 per cent ethnic Hungarians, most of them housed in the huge blocks of flats characteristic of the Ceausescu era". He does not say that the Hungarians were expelled from their lodgings, which were then destroyed by bulldozers, and their former inhabitants were forced to live in "the huge ugly blocks".

Ceausescu was a terrorist who practised genocide and had a terrible hatred of Hungarians. It is this type of sentiment that caused the terrible tragedy in Tîrgu Mures: the killing of unarmed Hungarian civilians, the wounding of more than 300 Hungarian civilians, the putting out of the eye of Suto, the well-known Hungarian writer, and other atrocities. Nothing similar occurred during more than 1,000 years of Hungarian rule.

The Hungarians of Transylvania are demanding only to be able to live freely without oppression and in harmony with the other ethnic groups.

ZOLTAN OVARY

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More on the Shroud

SIR—Regarding the procedure for obtaining samples from the Shroud of Turin for radiocarbon testing, M. S. Tite of the British Museum (*Nature* 332, 482; 7 April 1988) says, "all stages will be fully documented by video film and photography". However, the official report of the test results (*Nature* 337, 612; 1989) says: "All these operations, *except* for the wrapping of the samples in foil and their placing in containers, were fully documented by video film and photography" (my italics).

The key word here is "except"; its presence raises serious questions. Did the custodians of the Shroud agree that "all stages" of the procedure would "be fully documented" as Tite indicates in his letter of 7 April? If so, why did they make and suddenly break that reasonable agreement? (Note: The sampling of the Shroud took place on 21 April — just two weeks after Tite's reassuring letter appeared in *Nature*.) Why wasn't the entire procedure documented on video film and made available to the public? Without full documentation how can one be certain that it was the Shroud — and not another cloth — that was carbon dated? At what point in the sampling and sealing procedure did filming end — and why was it ended?

As Tite was the independent overseer of the carbon dating test and the guarantor of its unequivocal reliability, he has an obligation to address these unanswered

questions. (That obligation would be very apparent had the Shroud been carbon dated to the first century.) And it should be noted that the man of the Shroud and the person(s) who imprinted his Christlike image are, as yet, unidentified — and not everyone is convinced that the Shroud is mediaeval as the results of carbon testing indicate.

ROBERT HALISEY

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TITE REPLIES—I confirm that, as stated in the *Nature* article, the wrapping of the samples in foil and their placing in containers was not documented by video. This was because we were continuing to follow the blind testing procedures according to which only the Cardinal, Professor Gonella and myself were to know which containers held the Shroud samples.

This aspect of the procedure was, I admit, somewhat illogical, as by this time we were aware that, because of the unusual weave of the Shroud, blind testing was not feasible without unravelling the samples. However, I should emphasize that it was the Cardinal and myself who were guarantors of the samples and that the video film was intended as an *aide-mémoire* rather than being meant to provide definitive proof of the identity of the samples.

M. S. TITE

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HUGO's finances

SIR—We are, of course, delighted that the Howard Hughes Medical Institute (HHMI) has made a grant to HUGO of a substantial sum — \$1 million over four years. However, your report (*Nature* 345, 100; 1990) that this was HUGO's "first funding of note" is simply not true and we would like to set the record straight.

Earlier this year, you yourselves noted (*Nature* 334, 5; 1990) that the Wellcome Trust (not Burroughs Wellcome) had made a three-year grant to HUGO, to cover the costs of its London office and its European activities. The Wellcome Trust's award is for approximately £175,000 in the first year (with another £50,000 available for programme activities yet to be defined) and will, overall, be of about the same order of magnitude as the HHMI grant.

WALTER BODMER
(President)

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Global leadership in semiconductors

Hajime Karatsu

Success in the semiconductor industry requires bold investment in new technology and total commitment to quality. US companies will never catch up with their Japanese counterparts until they learn these and other lessons.

WHAT is the secret of Japan's success in the semiconductor industry? In the early 1970s, the world market for semiconductor products was dominated by the United States. But by the 1980s Japan had taken over the key sectors of the market (see figure over) and now, for example, supplies about 80 per cent of the world's memory chips. If the United States is to compete once again, fundamental changes in the attitude of industry are needed, from the top levels of management down to the shop floor.

Technological progress

The key to the next generation semiconductor market is investment bold enough to keep up with the pace of technological progress. The success of Toshiba, which has won a large share of the market for 1-megabit DRAM (dynamic random access memory) chips, demonstrates how investing with vision can put a company ahead of the competition. Toshiba started heavy investment in its semiconductor division in 1984 and managed to supply the 1-megabit DRAM chips two years before its competitors. With this foothold, the company could sell at a profit while taking rapid depreciation on the advanced equipment needed for production, and thus was better positioned than the late starters. As the result of this foresight, Toshiba's semiconductor business reportedly accounted for half of its profits of \$1.6 thousand million in 1989, even though the company is a highly diversified electronics giant with products ranging from locomotives to washing machines.

Today's fierce competition means that tomorrow's technology is being developed with ever-increasing speed. Adapting to this lightning pace requires aiming investment at the advances of the future. Toshiba's investment strategy was at least one year ahead of that of its competitors.

Shortening the time taken to develop new products is also critical. In the semiconductor industry, technological improvements are made in rapid succession, and introduced just as quickly to the shop floor. The old technology cannot match the new in cost and performance. Failure to adopt new technology will mean lost profits further down the road, and the longer the delay the greater the loss.

This may be why semiconductor firms in the United States have lost competitive ground to their foreign counterparts. A

short-sighted focus on quarterly profits runs counter to the nature of the semiconductor business.

Commitment to quality

The term 'learning curve' is losing its significance in the semiconductor industry as a result of refinements in manufacturing technology during the past decade: we are now able to derive high yields from new chips in the first period of production.

One reason for this progress is the concept of 'super clean' technology, developed in Japan at Tohoku University. The concept is simple: it involves striving for perfect cleanliness at every stage of the production process. Gas and materials are pure; machinery, pipes, wells and lighting do not generate dust; and workers are free of contamination. Production yields have improved dramatically where this approach has been adopted.

Almost all materials and equipment used today, however, fall short of these standards. In the 5 February issue of *Business Week*, an article entitled "The Future of Silicon Valley" contained a photograph showing a cloakroom in a semiconductor factory and careless handling of workers' clothes. In Japan, workers' clothes are washed every time they are used. When I was at Matsushita Communication Industrial Co., workers making engine control units for automobiles were required to change their gloves every three hours to prevent dust from entering the most high-precision stage of the process. Thanks partly to this precaution, the defect rate was less than 0.1 per cent.

Manufacturing is by its nature a continuing battle against error and uncertainty. This is doubly true for the semiconductor business. Procured materials might not fit the specification exactly, skilled operators might get sick, machines can perform unevenly, designs may have to be changed slightly at the request of customers, and a microscope might reveal bacteria on a chip.

Some engineers tend to think about manufacturing flaws in terms of black or white, but the 'grey areas' can be important. Forcing distinctions may mean that the root of the problem is never reached.

At Matsushita, we received some defective products from a factory in Singapore that was affiliated to a US company. In one package, we found that contamination on the chips had corroded the aluminium pattern. When the factory manager

visited Japan, I asked him to be more careful about his workers' clothing and masks and to review procedures for protecting against contamination. He replied that his men applied passivation covers to protect the chips from dust, so there must have been some other cause for the defect. The factory manager would not budge from his viewpoint. His attitude could be paraphrased as follows: "We tried as hard as possible. Why are the Japanese complaining about trifles?" I wonder what the response would have been if such defects had been found in a Japanese factory. Because dust is intrinsically harmful to semiconductors, all employees would have redoubled their efforts to prevent a recurrence, even if passivation covers had been applied.

Reaching a decision without exploring the middle ground, as the factory manager did, is not conducive to solving problems. This syndrome is exacerbated by confining employees to their specialized fields, where they are likely to develop a compartmentalized, self-serving attitude that may inhibit them from admitting their own mistakes. Attitudes such as that of the factory manager will not help to improve production efficiency and/or quality. Even if errors can be explained away, the product has the final word.

There are three ways to address the grey areas of manufacturing flaws. The first is to improve robustness against variation or error in production by selecting optimum parameters for materials, components and machine settings during design engineering. A new design methodology that has recently attracted attention is the Taguchi method, developed by Dr Genichi Taguchi of Bell Laboratories, for designing memory chips. It is gradually gaining favour in the United States and Japan. The second is to make the grey areas as small as possible by using 'super clean' technology. The third is aggressively to seek out sources of manufacturing errors that cannot be totally eliminated — that is, total quality control.

'Bottom up' strategy

Companies in Japan and the United States tend to have fundamentally different approaches to commercializing new technology. To borrow a metaphor from America's favourite pastime, US firms try to hit home runs, whereas Japanese companies try for single and squeeze plays. When carbon fibre was developed, for

example, US engineers applied the technology to aviation, envisaging halving aircraft weight. But a new technology such as this tends to be accompanied by defects, requiring a long time for quality to be assured. So Japanese companies started using carbon fibre for making lightweight golf-club shafts and fishing rods, products in which defects were more easily corrected. Because of the mass market for these products, manufacturing volume increased rapidly, quality improved and costs declined. With this experience behind them, companies began applying carbon-fibre technology to aircraft. Today, the Japanese control 60 per cent of the global carbon-fibre market. I call this approach the 'bottom up' strategy.

The difference in approach to technology commercialization is also evident in LSIs (large-scale integrated circuits). Companies in the United States began by using them in sophisticated applications, such as airborne equipment, missiles and computers. Japanese companies, on the other hand, first used the chips in small calculators, leading to the development of pocket models. At the time, the cost of LSIs was too high for the consumer market, but Japanese engineers correctly perceived that it would decrease quickly as a result of widespread demand for more-compact calculators. When the models were first manufactured, an LSI chip cost \$70; today, it costs only \$1.

A critical factor in the development of pocket-sized calculator chips was CMOS (complementary metal-oxide semiconductor) technology. Japanese companies concentrated on this technology to lower the power consumption of calculators and wristwatches. This was the key to the development of random-access memory chips.

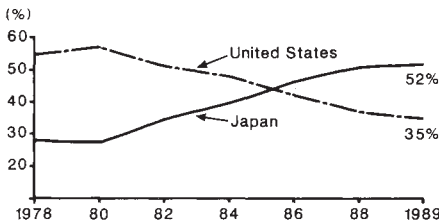
Another factor supporting Japanese companies' competitiveness involves the US-Japan semiconductor agreement, instigated by the United States. The anti-dumping provisions in the agreement drove market prices to double or triple. As Japanese semiconductor manufacturers also make computers, domestic appliances and electronic components, their electronics products were protected from price rises. Because intra-company shipments are not covered by the agreement, the parties harmed most by the sanctions were the US companies that depended on outside chip supplies. In effect, it was the goose that laid the golden egg, bringing in an enormous amount of money, which was in turn reinvested to further advanced semiconductor technology.

Defence market

Compared with the fierce consumer market, the defence market is rather sedate. At first sight, it would seem to be attractive because it demands the most advanced technology with less regard to

cost, and the government guarantees the supplier's profit. When semiconductor technology was being developed, support from the US Department of Defense was important in making possible large investment for research and development. Some key technologies were developed as a result of support for defence projects.

But these merits conceal a pitfall for the semiconductor industry. Defence applications often do not keep up with the fast pace of technological progress. Once a chip is installed in a specific weapons system, the providers are requested to



US and Japanese shares of the world semiconductor market (on the basis of sales).

supply it for at least the next ten years. Any modification or improvement is not allowed in the name of standardization.

In the civilian market, by contrast, companies jockey for competitive position by continually introducing improved technologies. Inferior products are forced out of the market. In Japan in 1989, 115 new facsimile models and 96 new copier models were released, attesting to the effects of stiff competition in the consumer and business markets.

It should also be borne in mind that production volume for consumer use is always greater than for defence use. Take, for example, the laser diode, a key component of compact-disc players. Hundreds of thousands of these diodes are manufactured every month in Japan. Technology developed for the mass market often has defence applications: CCDs (charge-coupled devices) for camcorders — produced in the same volume as laser diodes — are also used in the 'smart bomb', where they act as guides to the target.

In Japan, a stream of sophisticated new technologies superior to those created for defence use have been developed under the stimulus of free competition. The use of such advanced components is expanding the consumer market. In Japan, an auto-pilot model airplane equipped with a microprocessor and gyroscope costs about \$300 in hobby shops, and robot toys are very popular with young people.

High-definition television (HDTV) is one of the hottest technologies of the next generation, even though work on it started more than 15 years ago at the research institute of the Japan Broadcasting Corporation. Since last year, one hour of test HDTV is broadcast every day by satellite. Meanwhile, Japanese manufacturers of domestic appliances are investing

large sums to develop both new chips and display technology, including liquid-crystal flat displays (LCD). Japanese electronics companies began developing giant-size flat-screen display technology, for which their expenditure for constructing LCD factories is estimated at \$10 thousand million last year. Wali-type TV screens should be on sale in Japan within five years. HDTV technology also has important defence applications, and is sure to be transferred to the military field — Japan has already supplied HDTV equipment to the United States for possible use in the strategic command room.

Attracting talent

In 1987, I attended a conference in Finland at which a vice-president of McDonnell Douglas lectured on the next-generation transport aircraft. Through the introduction of such technologies as a nonducted-fan jet engine and a low-friction wing, he said, the aircraft will consume only about one-third the fuel of today's transports. These projects are sponsored by NASA.

As I listened to him speak, I imagined myself as a graduate of a US engineering school and asked myself if, with such exciting innovations in the aircraft industry, I would choose to work for the automobile industry, which is battered by overseas competition? I would opt for the aircraft industry, of course, and that is a cause for concern, because any industry that cannot attract talented engineers is heading for decline.

During a break, I asked the McDonnell Douglas executive about the size of the aircraft industry. He replied that it had \$80 thousand million in annual sales. Sales in the US automobile industry total \$270 thousand million. Given the strategic economic importance of the automobile industry, it has to attract the best engineers. The iron and steel industry lost much of its competitiveness because management, protected by quota systems, failed to adopt new technology. It thus became an undesirable sector for young engineers. The size of the steel industry is about \$30 thousand million, just half that of the aircraft industry. The economic consequences would be much more severe if the automobile industry suffered the same fate.

I cannot stress too much the need to make those industries fundamental to a nation's economy attractive to young graduates. In Japan, we have been fortunate to have been successful in drawing many of the brightest graduates to work in them. But all industrial nations could do better in that respect, not least in the United States. □

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Stefan Marinov's seasonal puzzle

With yet another holiday season brewing up in the Northern Hemisphere, readers may wish to brood on a conundrum devised by an anti-relativist. No prizes are offered for a solution.

STEFAN Marinov, the exiled Bulgarian physicist now living in Graz in Austria, is himself a puzzle. He is indefatigable in the prosecution of what seems to be his only cause, which is to prove that Einstein's theory of relativity is a pack of lies.

Marinov claims, among other things, to have shown by direct measurement that the velocity of light differs according to the direction of its travel along a fixed path. He has also partly developed what he says is a perpetual motion machine, a kind of Wimshurst machine driven by an electric motor which, when last inspected at this journal's London office, was said to require a car battery for its operation.

But most of Marinov's work is theoretical. Over the years, he has bombarded this and other journals with a series of papers with titles such as "The myths in physics" and "Violations of the laws of conservation of angular momentum and energy". Details of his correspondence with several editors may be found in his series of volumes called *The Thorny Way of Truth*, of which seven volumes have now been published by International Publishers (East-West) at Graz, of which Marinov appears to be the sole owner.

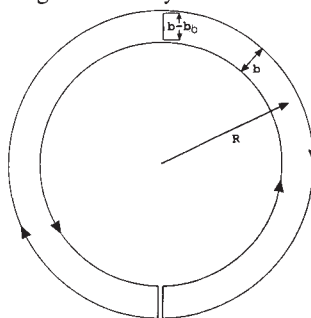
(From time to time, Marinov threatens to immolate himself in front of a British embassy or consulate if he cannot get an answer to a question about the publication of one of his papers. Some years ago, when embarked on such an enterprise outside the British consulate at Genoa, Marinov ran away when the police approached. Afterwards he explained that he had done so because he was in Italy illegally, without a visa. Mercifully, he has not threatened to immolate himself on *Nature's* account for many months.)

Part of everybody's difficulty is that too little is said about the details of the experiments whose results are said to contradict common expectation for even sympathetic critics to be able to provide constructive statements of how they and Marinov part company. Another is that Marinov's single-minded zeal for his own conviction considerably outstrips the zeal of those who may disagree. But from time to time, in Marinov's copious writings, there are relatively simple arguments that appear accessible even to those still at high school. Here is one series of *gedanken* experiments presented as if it were a Christmas puzzle (the original intention), with some helpful (or misleading) hints for its solution.

The figure shows a pair of circular con-

ductors arranged as two concentric circles. Equal electrical currents are circulated in each, but in opposite directions. The simplest way of creating this arrangement is to cut through the concentric pair at some point and to join the loose ends in pairs by short lengths of straight conductor. An electromotive force applied anywhere along the conductor will engender a current which must be everywhere uniform. At the bridged gap, there will be equal currents flowing in opposite directions, so their influence on the magnetic fields in the concentric gap will be zero.

The device is thus a means of arranging that there is a uniform magnetic field in the space between the concentric circles in a direction perpendicular to their plane (downwards into the plane of the paper when the current in the circuit flows in the direction indicated). The sensor in the experiment is a conductor long enough just to bridge the gap between the concentric circles and mounted on thin insulating supports in such a way that it can be made to slide around the circle. The objective is to measure the voltage across the sliding conductor, either by a standard voltmeter or by a condenser whose accumulated charge will be a measure of the voltage in a steady state.



The simplest case is when the sliding conductor is at rest. Then there is no voltage. Right? Next comes the case in which the sliding conductor moves at uniform speed around the concentric gap, always pointing along a radius of the concentric circles. As the slider moves, it will cut through magnetic lines of force at a constant rate, so there will be a constant voltage across the ends. The polarity of the slider will depend only on the direction of the current in the concentric circuit, and not on whether the slider moves clockwise or anticlockwise. Right again?

Now comes the tricky part, at least so far as Marinov is concerned. What happens if the sliding conductor is fixed in space, but the underlying concentric cir-

cuit is rotated about its centre? Relativity theory naturally predicts that the voltage across the sliding conductor would be the same as in the first experiment, and with the same polarity. On the other hand, questions may be raised about the degree to which the pattern of magnetic forces generated by the current is dragged around the ring by its rotation. Maybe there is a smaller voltage, but with the same polarity. What, asks Marinov, is the answer?

The second conundrum is superficially simpler: simply rotate the apparatus in its own plane, about the centre of the concentric circles. (There will be a small voltage due to the Earth's magnetic field, but this may safely be neglected.) Is there now a voltage, and with what polarity? If the answer to the first question is "Yes" the answer to the second must be "No", and vice versa. Readers are invited to make up their minds before reading on.

Marinov's own answers are unambiguous. Vice versa wins the day. When the underlying concentric circuit is rotated and the slider is kept fixed, there is no voltage across the movable conductor. But when the whole apparatus is rotated about its centre, the voltage across the now-moving sliding conductor is identical with that obtained when the slider is moving relative to the concentric circuit.

The implications are evidently important. The null answer to Marinov's first question implies that relativity has vanished through the window, the affirmative answer to the second implies that an isolated apparatus carrying a circulating current will generate a voltage when rotated, which raises forbidden questions about absolute space. Indeed, Marinov has devised a *gedanken* measurement of the Earth's velocity through space by stretching his concentric circuit into a linear version of it. He also claims that these violations of simple expectation are the basis on which his perpetual motion machine is built.

What is the truth? Nobody is quite sure, for nobody has done the experiments — not even Marinov. Indeed, one's confidence in the whole enterprise is somewhat undermined by Marinov's flat statement that he does not bother to repeat experiments whose outcome must be obvious. For one whose confidence in his own heterodoxy appears to be sustained only by his confidence that experiments will prove him right, Marinov seems curiously passive in the business.

John Maddox

A chaotic synthesis

Rory Howlett

ECOLOGY has now emerged as an exact science¹. This was the message of a recent meeting on the regulation and relative abundance of plant and animal populations*, an area where synthesis is not only possible, but probable (R. Southwood, Oxford University).

A growing trend in ecology is the analysis of long-term datasets to study the dynamics of populations either in isolation or when interacting with other species or variable environments. A common observation is that the apparent variability of population densities increases when calculated over longer periods of time². Whereas plant populations have rather tame, often successional dynamics with recruitment depending upon the death of dominant plants (M. Crawley, Imperial College at Silwood Park), animal populations exhibit a vast array of dynamic behaviours, including periodic cycles and apparent chaos (I. Hanski, Helsinki University). Much attention is paid to the factors underlying population regulation, defined as long-term persistence and fluctuations within limits, the lower limit being greater than zero (see Murdoch and Walde in ref. 1). Comparisons within and between taxa show that species tend to have more constant populations when there is some dependence of *per capita* growth rate on population densities. But such density-dependence, although apparent in some long-term time-series, is generally difficult to detect, even when it is assumed to be operative. Moreover, as Hanski points out, local populations tend to be embedded in more broad-scale 'meta-populations' — dynamic entities in which local populations can become extinct only to be re-established later by dispersal — and this will critically affect their local dynamics.

The difficulties involved in detecting density-dependent regulation of animal populations were highlighted by Anne Keymer (Oxford University), whose group has been grappling with the ecology of human helminth infections. These parasites are generally highly aggregated among hosts and the overall populations tend to be temporally stable. Furthermore, interventions designed to control disease have resulted in large-scale population perturbations from which recovery is often rapid, suggesting strong regulation. But the actual density-dependent mechanisms are hard to pin down. They may arise from competition among parasites within hosts when at high density, or from acquired immunity of hosts after

long exposure to parasites. Keymer argued that the latter mechanism appears, on present evidence, to be the more important.

Populations and communities are often very complex, but the very nature of this complexity can sometimes provide important insights into regulatory processes. As pointed out by Peter Chesson (Ohio State University), populations have internal structures that stabilize them and favour the co-existence of competing species. Spatial heterogeneity in

squared (CV^2) of the density of searching parasitoids in the vicinity of each host exceeds approximately unity'. When this criterion is applied to the real datasets and partitioned into density-dependent and -independent components, it suggests that, for 9 of 34 published studies, heterogeneity is large enough to stabilize dynamics and that density-independent heterogeneity is often the main stabilizing factor.

One of the most exciting areas of population ecology is the application of techniques developed to investigate the dynamical behaviour of complex systems (H.C.J. Godfray, Imperial College at Silwood Park; S.P. Blythe, University of Strathclyde). Recently, ideas have been

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Tropical deforestation — ecologists are in a battle against time to understand the dynamics and diversity of the world's most complex and intractable ecosystems.

the environment also affects population dynamics profoundly. This theme was expanded by Peter Kareiva (University of Washington), whose previous work on the dynamics of ladybirds and their aphid prey in fields of goldenrod has shown that the dispersal behaviour and demography of interacting species play crucial roles in determining the response of populations to mosaic environments (see ref. 3). In the case of host-parasitoid interactions, patchiness in the environment can be stabilizing (M. Hassell, Imperial College at Silwood Park; S. Pacala, University of Connecticut). Theory suggests that this effect comes from variation in the amount of parasitism from patch to patch. Parasitoid attack rates can vary between patches in ways that may or may not depend on host density, but the dynamical consequences of this variation are difficult to assess. But recent work shows⁴ that the stability of host-parasitoid systems can be predicted using the simple rule: 'overall population densities will remain roughly steady from generation to generation if the coefficient of variation

developed for distinguishing measurement error from chaos in ecological time-series (G. Sugihara, Scripps Institution of Oceanography; see ref. 5); these have been successfully applied to data on measles, chickenpox and marine phytoplankton populations. But complexities arise when time series are subject to secular trends or darwinian evolution — an important consideration when analysing data such as those available for fish populations under heavy and sustained fishing pressure (J. Sheppard and D.H. Cushing, MAFF Directorate of Fisheries, Lowestoft). Indeed, the interplay of population dynamics and the evolutionary process is expected to influence population persistence (J. Travis, Florida State University), and even trends in biological diversity over geological time (J. Valentine, University of California, Santa Barbara).

Of all ecosystems, tropical forests must rank among the most complex. The challenge of understanding them has been taken up by S. Hubbell (Princeton University) and his colleagues, who have

* Regulation and relative abundance of plant and animal populations, The Royal Society, London, 23–24 May 1990.

collected vast amounts of data on spatial patterns of sapling recruitment in a 50-hectare plot of a neotropical forest on Barro Colorado Island, Panama. They have used these data to study how tree species diversity is maintained. Strong negative conspecific effects on sapling recruitment in some of the densest species may prevent them from completely dominating the canopy, but many species do not show these effects, and Hubbell concludes that they may play only a limited role in determining the dynamics of a forest ecosystem. Studies of similar plots of forest located at sites right around the tropics, Hubbell hopes, will allow the development of a 'statistical mechanics' of

complex communities.

Despite these advances, great gaps remain in our knowledge of even basic ecological facts, such as the total number of species on the planet (R.M. May, University of Oxford and Imperial College, London). Only through understanding the structure and regulation of communities can we hope to plan the conservation of life's diversity. □

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OPTICAL ASTRONOMY

Are spiral galaxies opaque?

Michael Disney

ASTRONOMY is bedevilled by the presence of clouds of obscuring smoke mixed with the gas that drifts between the stars. This smoke (or dust, as it is more widely, but incorrectly, called) cuts off from sight much of the Universe and makes it difficult for us to interpret what we do see. On page 153 of this issue¹, Valentijn presents evidence that spiral galaxies are not, as has long been supposed, transparent, but are heavily clogged up with their own interstellar smoke. If he is right, rethinking of some major issues will be required.

If, as Valentijn suggests, we are seeing only the outer crusts of galaxies, we cannot so glibly assume that exotic 'dark matter' is necessary to explain the rotation curves of spiral galaxies (that is, their rotation seems to imply that more mass is present than is inferred from their optical radiation). At least in part, the hidden matter may instead simply be stars buried beneath the smoke. And the 'hidden starbursts' that have been invoked² to explain the surprisingly large infrared luminosities of spiral galaxies seen with the IRAS satellite may not be so widespread — the infrared could be merely starlight from obscured stars, reradiated by the cold smoke. We will also have to reassess the true proportion of galaxies undergoing nuclear activity, currently thought to be one or two per cent, as we may be seeing only the least obscured nuclei. If, furthermore, galaxies really are opaque, then as they pile up in the distance and fill our field of view, they will block out the more distant Universe at optical and ultraviolet wavelengths, cutting off high-redshift quasars and spoiling our view of galaxy formation.

The smoke in our own Galaxy strongly affects what we can see of it. On a moonless night the dark-adapted eye can see

dark clouds of smoke outlined against the Milky Way. Optical radiation from the Galactic nucleus is attenuated by no less than 18 orders of magnitude. Fortunately, however, we live in the outer, relatively unobscured periphery of our Galaxy, so that if we look away from the plane of the Milky Way we get a fairly unobscured view of the extragalactic Universe. Astronomers in the majority of solar systems would not be so lucky, being forced to rely on longer-wavelength infrared and radio radiation to which smoke is more transparent.

When we observe another galaxy we have somehow to allow not only for obscuration along our line of sight but also the internal extinction within that galaxy itself due to its own smoke. How might this be done? In a highly influential paper in 1958, Erik Holmberg³ suggested that we compare the properties of spiral galaxies tipped at different angles to our lines of sight. If they are perfectly transparent, their luminosities will be the same in all directions. But if they are perfectly opaque, they will look brighter when seen face-on, thus subtending the largest area of sky. Holmberg devised and carried out the classical test for opacity in spiral galaxies by comparing their apparent luminosities (or surface brightness) with their inclinations to the line of sight. From that test he and his successors concluded that spiral galaxies are largely transparent: when seen face-on, less than 40 per cent of their light is extinguished by internal obscuration⁴.

Although simple in principle, Holmberg's test is very difficult to carry out in practice. How can one be certain, for example, that the edge-on galaxies in one's sample are drawn from the same intrinsic population as the face-on galaxies? One may not be comparing like with

like. And even if such selection effects can be discounted, one cannot infer the extinctions from the observations without first adopting some more or less arbitrary form for the distribution of the smoke. If sunk in the galactic plane or gathered into compact clouds, it will obscure less than if it were widely dispersed. In any case, optical observations suffer from a systematic bias: more light will come from the least obscured parts of the galaxy, leading to a systematic underestimate of its extinction⁵. Most astronomers over the past 30 years have either ignored or discounted these difficulties and accepted Holmberg's view that spirals are largely transparent.

Such were the difficulties of observation in his day that Holmberg was forced to work with a sample of less than 200 galaxies. Taking advantage of the new automated and digitized microphotometers, Laubers and Valentijn⁶ have measured at least 10,000 bright spirals appearing on the European Southern Observatory Survey plates. It is from this sample that Valentijn now draws his conclusions. With thousands rather than tens of galaxies to work with, he can be more selective in choosing his samples and, by comparing different samples, can make some estimate of his own selection effects. He compares the surface brightness of 2,500 spiral galaxies with their inclinations deduced from the ratio of their major to minor axes. Unlike Holmberg's, Valentijn's galaxies fit perfectly the totally opaque model, and are certainly inconsistent with the transparent or even semi-transparent models.

Valentijn's results clearly do not agree with Holmberg's or with Heidmann's⁷. Why this should be so is not clear. Valentijn believes that it is due partly to the greater selectivity that he can afford, and partly to the more precise definition and measurement of surface brightness afforded by his instrument. In one sense Valentijn's result is almost too good to be true, because it implies that even the outer parts of spiral galaxies are optically thick — a surprising result given the small amounts of gas detected there. To confirm the result requires mapping of spiral galaxies in the infrared, where they should be optically thin, and in the difficult sub-millimetre region, in which most of the cold (≈ 20 K) dust should

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show up in emission. Valentijn's result is more likely to stir up than to end the controversy.

If spiral galaxies are opaque, astronomers, and cosmologists in particular, will need to revise some of their ideas. Indeed, Davies⁸ and Valentijn¹ have both now pointed to the difficulty of naively inferring the existence of missing mass from optical photometry (although they

by no means discount the possibility of dark halos), and Wright⁹ has demonstrated recently that the decline in quasar numbers at high redshift can be plausibly accounted for by the presence of opaque galaxies in the foreground. □

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VISUAL NEUROSCIENCE

A causal chain in motion

Andrew Parker

EVER since microelectrodes reliably picked up action potentials from sensory nerve cells, there has been a continuing debate about the relationship between the firing patterns of cells and the perceptual consequences for the organism. In the early experiments, the emphasis was on simple detection of sensory stimulation¹⁻⁴. This work produced some elegant and exciting conclusions. The retinal ganglion cells, whose axons communicate with the rest of the central nervous system, can detect flashes of light so weak that they contain only a few quanta of light^{5,6}. The quality of sensation evoked by stimulation of single visual and tactile afferents may fall into discrete and readily identifiable perceptual classes^{7,8}. As shown by Salzman, Britten and Newsome on page 174 of this issue⁹, the same questions are being asked nowadays of regions of the brain that are more evidently concerned with higher-order perceptual analysis.

One suitable candidate region is the cortical visual area, V5 (or middle temporal, MT), whose function has been linked to the higher-order processing of visual motion¹⁰⁻¹². Individual cells in this area are selectively excited by particular directions of visual motion. Last year, Newsome's group at Stanford showed that the sensory thresholds of single neurons in MT could match, or even exceed, the behavioural performance of the animal in a motion discrimination task¹³. Moreover, on occasions, there can be a reliable trial-by-trial association between the firing of single neurons and the behavioural response¹⁴. Now, with another clever shuffling of classical techniques, the same group⁹ has studied the application to cortical neurons in MT of a weak, spatially localized, electrical stimulation at exactly the moment that a perceptual decision is required. Such a stimulation must slightly alter the firing patterns of MT cells and, as might be hoped, it can bias the perceptual decisions in a selective manner.

The experimental strategy relies on the functional organization of the properties of cells in MT. Not only is there a topographical map of the visual field onto this piece of cortex, but neurons with similar

selectivities for the direction of motion are clustered together — probably in some form of columnar arrangement. Salzman *et al.* carefully determined the motion properties of a small region of MT by analysing the excitation of neurons in response to visual patterns of moving dots. Next, they presented a target of moving dots restricted to the same patch of the visual field over which the neurons could be excited. The dots could move either in the direction locally preferred by the neurons or in the opposite direction.

The animal had been trained previously to signal its judgement of perceived direction by shifting gaze from the initial fixation point to one of two locations either side of the target. In order to push the perceptual decision to threshold limits, only a fraction of dots in the target actually moved in the designated direction. The remainder moved in random directions and the measure of motion stimulus strength was the percentage of dots moving in a consistent direction. The critical result is that local electrical stimulation, applied at the same time as the motion target, may add to the nervous system's estimate of motion stimulus strength in a direction-specific manner. The authors present evidence that the electrical stimulation does not directly elicit the eye-movement response that is used as a monitor of the perceptual decision.

There are two surprising features of this work. The first is that electrical stimulation of a very small region of the cortex significantly affects a perceptual decision of the whole organism. Perhaps stimulation affects only a few cells at the stimulation site or perhaps it excites a particular neural circuit serving motion analysis, but the result does imply an extreme localization of function. Indeed, certain cortical locations in MT seem to be especially favourable for eliciting shifts in perceptual decisions.

On the other hand, the applied stimulation is rather nonspecific in its temporal aspects. Although it was confined to the duration of the visual stimulus, there was apparently no need to match accurately any naturally occurring temporal pattern

of excitation for the cells. This gives little support to the notion that the exact temporal pattern of action potentials codes for the attributes of a stimulus. But this issue (and a number of related ones) can be resolved properly only when the experimental approach is modified to allow a more detailed analysis of the subjective qualities of sensation evoked by stimulation.

The information derived from this type of experiment should have at least one longer-term benefit. If the causal links between nervous activity and behaviour of the organism can be placed on a firmer basis, this knowledge will help to decide what kind of picture of brain activity is needed to assess neural function and dysfunction. The consequences for developments in brain-scanning techniques for use in clinical diagnosis could be profound.

It is an interesting comment on scientific fashion that 20–25 years ago the purist wing of artificial intelligence might have dismissed this kind of experiment as fatuous — after all, imagine trying to analyse the operation of a standard digital computer with a voltmeter, battery pack and a switch. Yet the brain clearly does not have a von Neumann architecture and has, through the course of evolution, generally adopted modular structures that have different functional properties, especially at a cortical level. Now the debate in parallel computing architectures is much concerned with structural organization and its consequences for computing efficiency, even at the hardware level. Although neural-network architectures are essentially parallel, their development has had separate motivations, often emphasizing the intrinsic value of distributed representations of information. How network systems can accommodate experimental results that imply highly localized and specific functional modules is somewhat problematic. □

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Ups and downs of African lakes

Neil Roberts

WHEREAS Quaternary climatic change in high latitudes is dominated by the buildup and decay of ice sheets, the dominant climatic signal in low-latitude regions is not glaciation but precipitation. In areas such as inter-tropical Africa, arid and humid phases have alternated as the monsoonal circulation system has been weakened and strengthened. Tropical palaeoclimate is recorded most clearly by changes in the water level and chemistry of 'non-outlet' lakes. These had low water levels, indicating an arid climate, during and after the last glacial maximum (21–12.5 kyr ago), followed by a period of high lake levels (a humid climate) during the early part of the present interglacial (12.5–5 kyr ago). Unlike the slow responses of glaciation and vegetation to high-latitude climatic change, many low- and mid-latitude lakes respond rapidly to hydro-climatic change. On page 141 of this issue¹, Gasse *et al.* present high-resolution stratigraphic records spanning the period from 15 to 7 kyr ago from two such 'responsive' lake basins, one on each side of the Sahara. These two records show that, like the eustatic sea-level record of Northern Hemisphere deglaciation², the last arid-to-humid transition in Africa was not smooth, being broken by climatic events lasting 100–1,000 years.

Major climate changes driven by the Milankovitch cycle of the Earth's orbital variations are well established. Sub-Milankovitch climatic oscillations (known traditionally as stadials and interstadials) superimposed on these longer-term astronomically induced variations have also long been recognized, but they are currently becoming of increased palaeo-environmental interest³.

Their causes remain a subject of debate, but these almost certainly include agencies such as major emissions of volcanic dust and the formation of North Atlantic deep water^{3,4}. Of these palaeoclimatic oscillations, the so-called Younger Dryas event (11–10 kyr ago) has received the greatest attention. Evidence now suggests that this event was felt well beyond its place of origin in northwest Europe, although its manifestation varied regionally across the globe^{5,6}.

In inter-tropical Africa, the period of the Younger Dryas is marked by sharp regressions in the water level of lakes such as Bosumtwi (Ghana, 6° N), Chad (13° N) and Ziway-Shala (Ethiopia, 8° N), indicating negative hydrological balances (a net deficit of water) and an arid climate⁷. These same lakes also record later regression (arid) events of apparently similar magnitude and duration to the one contemporaneous with the Younger Dryas,

for example between 8.0 and 7.2 kyr ago. This 7.5-kyr event is broadly coincident with, and may have been caused by, the collapse of the Laurentide ice sheet over Hudson's Bay. Although ¹⁴C chronologies indicate that the hydro-climatic events centred on 10.2 and 7.5 kyr ago were synchronous between different lake basins in Africa, they have seemed until recently to be geographically restricted to sites in the inter-tropical zone. But the investigation by Gasse *et al.* of the more

ling natural from cultural factors. Nonetheless, data are beginning to emerge which suggest that tropical climatic 'crises' occurred not simply over recent millennia but even in recent centuries. One especially startling piece of evidence comes from cores taken in Lake Malawi, which reveal that the level of this great lake fell by about 100 m at some stage within the past thousand years, signifying a reduction in rainfall at that time to 50–70 per cent of modern values¹⁰.

To understand the climatic mechanisms responsible for these hydro-climatic events, there is a need to establish as precisely as possible their spatial extent, age, duration and intensity. As Gasse *et*



H. F. Lamb

Lake Tigalmamine, in the Middle Atlas mountains of Morocco. Pollen and diatom analyses of sediment cores^{8,9} from this lake show evidence of abrupt short-term falls in lake level which occurred at times similar to those known from sites in tropical Africa.

northerly Sebkhla Mellala lake basin (Algeria, 32° N), and similar studies on the Tigalmamine basin (Morocco, 33° N) (refs 8 and 9, and H. Lamb *et al.*, unpublished work), now reveal that these short-lived regression events occurred beyond as well as within the tropics. The interruptions to the hydrological cycle at around 10.2 and 7.5 kyr ago are thus apparently of continental or even of global significance.

One intriguing question, on which the jury is still out, is whether sub-Milankovitch climatic events have occurred throughout the recent geological past or whether they were restricted to times of major climatic transition, such as the end of the last glaciation. Abrupt lake-level oscillations are not equally 'visible' throughout the late Quaternary record: regression events, for example, are stratigraphically much more evident at times of high rather than low lake levels. The record of the late Holocene (in any case a period of low lake levels in Africa) is complicated further by increasing human influence and the difficulty of disentangling

natural from cultural factors. Nonetheless, data are beginning to emerge which suggest that tropical climatic 'crises' occurred not simply over recent millennia but even in recent centuries. It is clear that, unlike the Milankovitch changes brought about by variations in the solar radiation received by the Earth, these events cannot easily be predicted, thereby creating considerable uncertainty for projections of future climate. □

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One neuron, many units?

Larry Cohen and Jian-young Wu

Is the neuron the functional unit of the nervous system or can small portions of a neuron act as independent units? Neurobiologists have been speculating about this second possibility for more than 30 years^{1,2}, partly because microelectrode recordings from cerebellar Purkinje neurons reveal small spike signals that are almost certainly all-or-nothing events occurring in restricted areas of the dendritic tree^{3,4}. Bill Ross, Nechama Lasser-Ross and Bob Werman⁵ have now determined the spatial distribution of such events by using multi-site optical recordings with a calcium indicator dye. The findings are important because they show that one can now directly measure the independent activation of parts of a neuron.

One of the results obtained by Ross *et al.* is represented in Fig. 1, which shows a high-time-resolution recording from a Purkinje cell in a slice from guinea-pig cerebellum. Three dendritic regions can be differentiated: a region on the lower left where both of the small spike signals are detected as rapid increases in calcium; regions on the upper left and lower right where only the second of these signals is detected; and regions where neither signal is detected. In one especially complex cell, the authors were able to resolve six different compartments.

Because the dendrites of the cerebellar Purkinje cell are postsynaptic only, the effects of independent activation of the dendritic elements would be integrated at the spike-initiating zone, thereby determining the cell's output. This kind of organization is not, however, universal. Other neurons have both output and input synapses in the dendritic tree⁶. If a dendrite of such a neuron were independently activated, it would form a complete functional unit. It is possible, for example, that dendrites function independently in certain horizontal cells and starburst amacrine cells of the retina, and in nonspiking interneurons in the locust. Calculations suggest that the dendritic trees of these neurons can be divided into several electrically distant regions⁷⁻¹⁰, and electron micrographs show that the independent regions both receive and make synaptic contacts. Furthermore, for both the horizontal cell⁷ and locust interneuron¹¹ there is physiological evidence that is consistent with the existence of independent units, but this has not so far been directly demonstrated. Such a demonstration might best be achieved by measuring the membrane potential at many sites using a voltage-sensitive dye on the inside of the neuron: changes in membrane potential may be interpreted more easily than changes in calcium concentration.

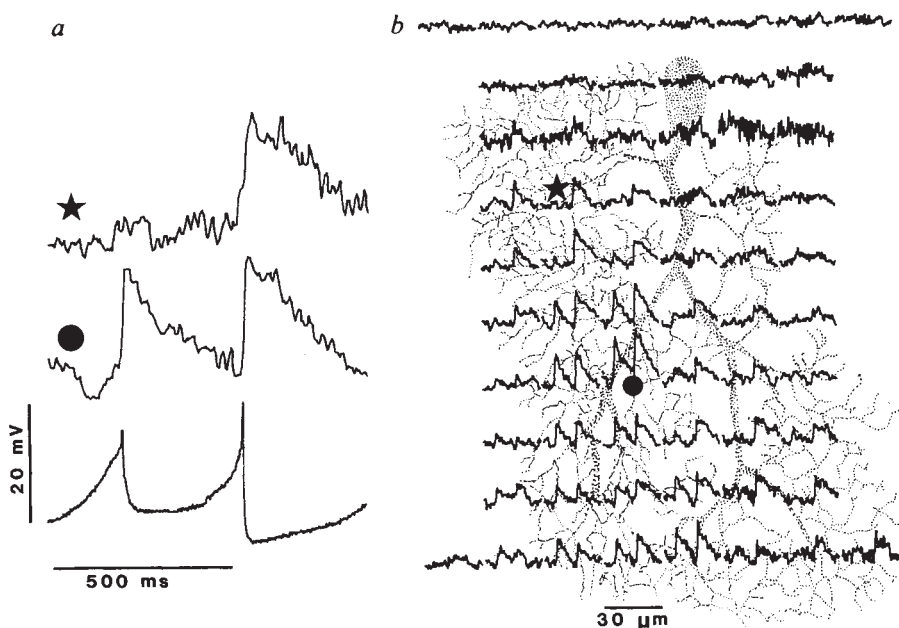


FIG. 1 Optical and microelectrode recordings from a Purkinje cell that has been microinjected with Arsenazo III in a 200-μm slice from guinea-pig cerebellum. *a*, The microelectrode recording from the soma (bottom trace) shows two small spontaneous spikes which differ in size. Optical recordings from detectors receiving light from two different areas of the dendritic tree are shown above the recording. In the top recording only the second spike event is detected, whereas in the middle trace both spike events are seen. *b*, Outputs of 64 photodiodes receiving light from 64 areas (each 30 × 30 μm square) of the slice overlaid on a drawing of the neuron. The cell body is at the top; the axon (not shown) leaves the cell body at the top of the figure. (Modified from ref. 5.)

Unfortunately, however, the signals from voltage-sensitive dyes applied to the inside of neurons have so far been relatively small^{12,13}.

It is difficult to predict how widespread independently acting dendritic units might be; there are neurons in which independent activity seems unlikely. For example,

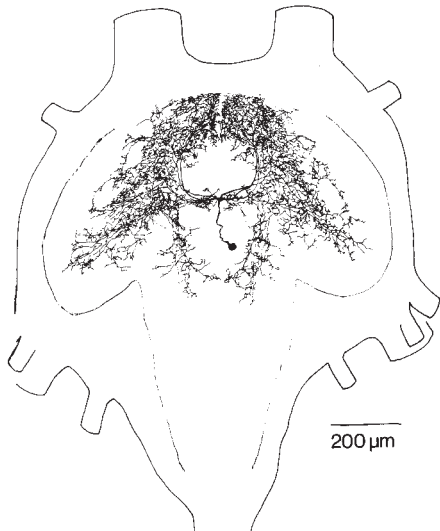


FIG. 2 Structure of a small dorsal unpaired median (DUM) spiking interneuron from a grasshopper ventral ganglion. The cell was stained with cobalt-silver and drawn from a whole mount. (Modified from ref. 19, courtesy of Karen Thompson and Melody Siegler.)

signals initiated anywhere in the electrically compact dendritic tree of an insect neuron can be detected equally in the cell body¹⁴. And in some crustacean neurons it has been impossible to detect compartmentalization of the calcium signal¹⁵.

Although the prospect of directly measuring independently functioning regions of the dendritic tree is exciting for cellular neurobiologists, the existence of such regions would pose a problem for those trying to understand how behaviours are generated from neuronal interactions. Monitoring the activity of independent units in a dendritic tree is much more difficult than simply keeping track of the membrane potential of the cell body.

This would not be the first instance where an important discovery in cellular neurobiology increased the difficulty of understanding how behaviours are generated. Another was the demonstration that some neurons do not generate action potentials; these nonspiking neurons maintain their resting potentials in the middle of the range of potentials leading to transmitter release^{2,6,16}. A problem in monitoring the activity of a nonspiking neuron is that the membrane potential measured at one location in the cell might not be an accurate representation of the potential at other locations in the cell.

Although nonspiking neurons are widespread in the animal kingdom³, there are nervous systems in which such cells are

relatively rare. Even though nonspiking neurons are found in gastropod molluscs^{17,18}, the best guess is that less than one per cent of the central neurons in *Aplysia* are nonspiking. Furthermore, nonspiking synaptic interactions are also rare in *Aplysia*.

A third instance of a double-edged success for cellular neurobiology is provided by the complex structure of neurons seen under the microscope. One beautifully ornate example is the interneuron from a grasshopper ventral ganglion illustrated in Fig. 2. The extent of the dendritic tree implies that the neuron may be in synaptic contact with a large number of other neurons in the ganglion. Obviously, a nervous system in which each neuron interacts with many neurons is more difficult to analyse than one in which synaptic interactions are restricted.

Widespread synaptic interactions may be the rule rather than the exception. For example, serial-section electron microscopy indicates that a mechanosensory cell in the leech makes about 2,500 anatomical synapses onto postsynaptic neurons²⁰ (K. Müller, personal communication); it would be interesting to know the distribution of the 2,500 synapses among the 390 neurons in the ganglion. In the nematode *Caenorhabditis elegans*, the ADE-L sensory neuron is monosynaptically connected to 9 per cent of all other neurons in the animal and disynaptically connected to an additional 52 per cent. Only slightly

lower percentages are obtained starting with sensory neurons OLL-L and PHC-L (compiled from ref. 21). Finally, in *Aplysia*, 300–400 of the 1,000 or so neurons in the abdominal ganglion are activated by lightly touching the siphon skin^{22,23}. It seems that information about sensory stimuli is propagated widely in these nervous systems. This raises the possibility that the organization of the response to the stimulus may also involve many neurons²⁴.

It will be exciting to see what roles these complex cellular properties have in the generation of behaviours. Using the EVOLUTIONARY EPIDEMIOLOGY

Parasite clones in the wild

Anne E. Keymer, Robert M. May and Paul H. Harvey

SEVERAL species of parasitic protozoa will reproduce sexually in the laboratory^{1–3}. That sexuality is an advantage in such cases is indicated by an excess of recombinants being produced from mixed-genotype infections⁴. It does not follow, however, that sexuality is important in the wild just because it can occur and have advantages in the laboratory. Indeed, preliminary field observations of several species (including the protozoa that cause leishmaniasis and Chagas disease) have indicated clonal population structures that are stable for many generations and over wide geographical areas^{5,6}. Until recently it could be assumed that the laboratory and field studies had looked at different species or different strains of a species, and that sexual reproduction differed in frequency between the two groups. But a new report by Tibayrenc and colleagues in *Proceedings of the National Academy of Sciences*⁷ casts doubt on this interpretation: species which may be sexual in the laboratory have a clonal population structure in the wild. The finding may have important implications for biological control programmes.

Using data from species belonging to seven genera of parasites, Tibayrenc *et al.* propose that clonal population structures are the norm in natural populations. In each genus (*Entamoeba*, *Giardia*, *Leishmania*, *Naegleria*, *Plasmodium*, *Trichomonas*, *Trypanosoma*) there are fewer genotypes than would be expected if regular outbreeding occurred. In their analyses of biochemical variability, the authors used seven criteria when testing for clonality. Three are based on tests of the significance of observed levels of segregation and are applicable only to diploid organisms: fixed heterozygosity, the absence of segregation genotypes, and deviation from the expectations of a simple Hardy–Weinberg equilibrium model. The other four criteria are tests of the significance of observed levels of

techniques of Ross *et al.*, the existence of independent functional units in a neuron can now be directly studied. It will be a challenge to systems neurobiologists to develop methods for handling the potential complexity of widely interconnected neurons. But, surely, the question of how behaviours are generated from interactions of neuronal elements is the central concern of neurobiology. □

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recombination and are thus independent of ploidy: widespread existence of identical genotypes, absence of recombinant genotypes, linkage disequilibrium, and correlations between independent sets of genetic markers. The results imply that naturally occurring populations derive from the clonal reproduction of a few highly successful genotypes, as highlighted by the predominance of particular clones in different localities, sometimes in different host species, and at intervals separated by many years.

A few examples illustrate the new findings. The *Leishmania* zymodeme (a genotype determined by electrophoretically distinguished enzyme patterns) MON 1 is predominant in both the Old World and in Latin America. A clone of *Plasmodium falciparum* (Gpi1/Ada1/Ldh1/Pep1) has been isolated from ten out of 17 samples in five countries spanning Africa and Indochina. *Entamoeba histolytica* zymodemes I, II and III have been sampled repeatedly in Canada and South Africa. Six identical strains of *Trichomonas vaginalis* have been identified: five that were isolated recently, and one in 1939. And a single zymodeme of *Giardia* has been found in five human hosts and two cats (one in Western Australia and the other in Oregon).

The results imply that clonality should be adopted as the working hypothesis by those involved in research on diseases caused by the parasitic protozoa. Genetic

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recombination is not ruled out — the data simply show that it does not significantly affect the prevailing patterns of clonal population structure. What, then, of the sexuality so clearly demonstrated in the laboratory? Are recombinants selected against in the wild?

More ambitiously, can these systems provide us with precise, quantitative insights into evolutionary problems such as the generation of stable or shifting polymorphisms by frequency-dependent selection, or the origins and maintenance of sexual reproduction^{8,9}? To answer

these questions, we need to know more about the epidemiology of individual clones, the frequency of multiple-clone infection, and the mechanisms and consequences of clonal competition. Increased integration of work in the field and in the laboratory will help, as will further cooperation between geneticists and epidemiologists. □

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SUPERCONDUCTIVITY

Bringing about some order

Robert J. Cava

It seems fitting that $\text{YBa}_2\text{Cu}_3\text{O}_x$, the first material found to be superconducting above liquid-nitrogen temperature, exhibits one of the most interesting and complex interplays between chemistry, crystal structure and physical properties of any ceramic material studied so far. At the heart of the matter is a subtle charge balance between the one-dimensional copper-oxygen chains, which have variable oxygen content, and the two-dimensional copper-oxygen pyramidal planes, where superconductivity originates (see figure). The latest surprise is described in an elegant paper by Jorgensen *et al.*¹, who find a correlation between the appearance of superconductivity in an originally nonsuperconducting oxygen-deficient sample, after annealing at room temperature, and time-dependent changes in its crystal structure. As well as demonstrating that the oxygen atoms are mobile at ambient temperature, the results show more importantly that the superconducting properties are remarkably dependent on local, short-range structure. This situation is likely to be unique to the high-transition-temperature copper oxides, in which the coherence lengths of the superconducting charge carriers are of the order of the size of the crystallographic unit cell.

In oxygen-deficient $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$, oxygen is removed from the Cu-O chains: a 90-K superconductor is obtained for $0 \leq x \leq 0.2$, a 60-K superconductor for $0.3 \leq x \leq 0.55$, and an antiferromagnetic insulator for $0.55 \leq x \leq 1.0$. These changes can be shown to correspond to a series of charge transfers from the chains to the pyramidal planes². In $\text{YBa}_2\text{Cu}_3\text{O}_7$, the Cu-O chains are aligned along one crystallographic axis (the orthorhombic b axis), while an equivalent direction in which they could lie — the a axis, perpendicular to the b axis — remains unoccupied (thus the a axis is shorter, as the chain oxygens are missing; see figure). As

oxygen is removed, the chains become fragmented; eventually the fragments become short enough to be able to align along either the a or the b axes. These axes then become equivalent, making the overall symmetry tetragonal even though on the scale of the length of the chain fragments the symmetry is still orthorhombic. The orthorhombic-to-tetragonal phase transition is a function of both the total oxygen content and the temperature, because the orientational disordering of the chain fragments increases the entropy of the system.

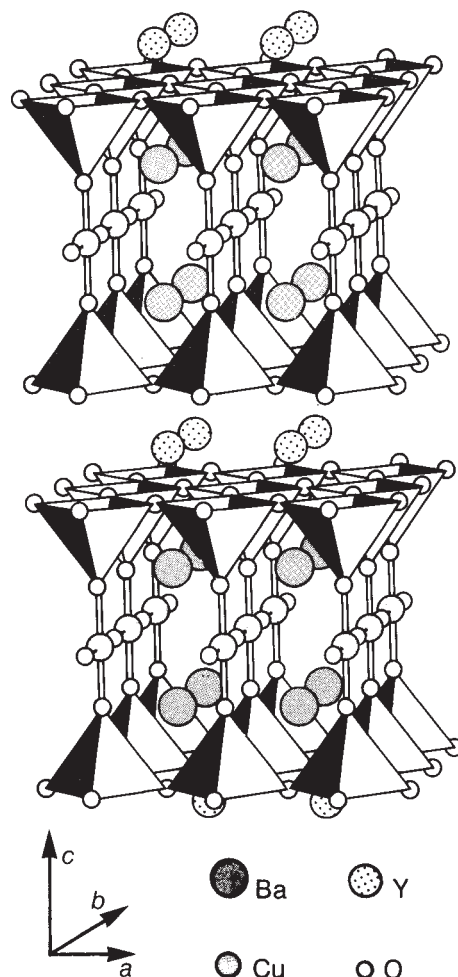
The paper by Jorgensen *et al.* follows up their initial discovery³ of time-dependent superconducting phenomena in $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$. The present results were obtained from polycrystalline samples prepared at the composition $\text{YBa}_2\text{Cu}_3\text{O}_{6.41}$ by annealing $\text{YBa}_2\text{Cu}_3\text{O}_7$ for a long period at 520 °C at reduced oxygen pressure and then quenching into liquid nitrogen. At the preparation temperature and oxygen content, the compound is tetragonal with disordered chain fragments. The quenching is performed to maintain the sample as much as possible in the high-temperature disordered state, which should not be stable with respect to lower-entropy states at room temperature. But the orthorhombic symmetry observed for the samples immediately after quenching indicates that even at a rapid quench rate the oxygen atoms have time to partially rearrange from the disordered structure.

One of the clever aspects of these experiments is that Jorgensen *et al.* pick an oxygen content right at the superconducting/nonsuperconducting boundary. The sample is initially nonsuperconducting, but soon develops a superconducting transition with a transition temperature that saturates at 20 K after about 72 hours. This temperature is significantly lower than the 55–60 K observed for 'equilibrium' samples at a composition near $\text{YBa}_2\text{Cu}_3\text{O}_{6.50-6.45}$ (which corresponds

to the minimum oxygen content generally required for superconductivity).

In a demonstration of one of the great assets of the neutron time-of-flight powder-diffraction technique, the authors have made full crystal-structure determinations at 27 stages during the first 26 hours of the ambient-temperature annealing. The structural changes involve the shortening of all the crystallographic axes, with the greatest shrinkage along the a axis, and also a shortening of the bond between the pyramidal copper and its apical oxygen. Surprisingly there is no change (within the precision of the measurements) in the amount of oxygen in the chain sites on the a axis relative to the those on the b axis, implying that there is no large-scale lengthening or increase in the number of chain fragments aligned along b at the expense of those along a . In addition, there is no change in the total oxygen content.

The authors estimate the length scale of the diffusion process that gives rise to these structural and physical changes to be 4 Å — approximately the size of a unit cell. The appearance of superconductivity, therefore, is due to the short-range



The structure of $\text{YBa}_2\text{Cu}_3\text{O}_7$, showing the one-dimensional Cu-O chains and the two-dimensional Cu-O pyramidal planes. Crystallographic a , b and c axes are also shown.

rearrangement of oxygen atoms in the chains, giving rise to charge transfer to the planes as is evidenced by the decrease in the distance between the plane copper atoms and the apical oxygen. Because of the very short coherence lengths of the superconducting charge carriers, superconductivity is observed even when only microscopic regions of the crystallites have the appropriate oxygen configuration. The relatively low transition temperature may be due to the proximity of superconducting and nonsuperconducting regions. It is not easy to understand, without more data, exactly how a microscopic rearrangement of oxygen atoms can produce no change in the overall ratio of the *a*- to *b*-orientated chain frag-

ments while disproportionately decreasing the *a*-axis length. Perhaps, as Jorgensen *et al.* suggest, it involves the formation of alternating, parallel full-chain/empty-chain fragments on a local scale, as are found on a longer scale for the 60-K superconductor $\text{YBa}_2\text{Cu}_3\text{O}_{6.5}$. But whatever the case, whereas some might argue that there can no longer be anything new or exciting to learn about $\text{YBa}_2\text{Cu}_3\text{O}_7$, Jorgensen *et al.* have clearly shown otherwise. □

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OCEAN DRILLING PROGRAM

Reading the ocean's diary

Leg 130 shipboard scientific party

How does sedimentation respond to changes in global climate and ocean dynamics? And what is the origin of the world's largest oceanic plateau? It was to address these questions that Leg 130 of the Ocean Drilling Program took the RV *JOIDES Resolution* to the Ontong Java Plateau in the western equatorial Pacific. This plateau is ideally suited to such studies, being a flat, shallow region of sea floor — the result of an unusually thick (40-km) oceanic crust and a thick (1,400-m) sediment cover. Being free of interference from coastal ocean processes, the region has the potential to provide a complete, undisturbed record of open-ocean pelagic sedimentation since the middle Cretaceous (about 100 million years ago). The cores recovered by Leg 130 succeeded not only in illuminating this history, but also in supplying clues to the origin of the plateau itself.

The Ontong Java Plateau (Fig. 1) has drawn the interest of marine geologists since the beginnings of deep-sea drilling^{1–3}, and has provided standard sedimentary sections for the evaluation of Pleistocene palaeoceanography^{4,5}. Two boreholes drilled during Leg 130 penetrated through the sediment cover into the basalt basement below (one to a depth of 26 m and the other to 149 m into the basalt), opening the way for petrological studies of the origin of the plateau and for palaeomagnetic studies of its movement since the middle Cretaceous. The basements of oceanic plateaus in the Pacific have been penetrated only four times by previous drilling projects, and never before to such depth. The Leg 130 results show that the Ontong Java Plateau is oceanic in origin, having been formed at depths of 2,500–3,000 m during the early Cretaceous from flood basalts and flows of basaltic pillow lavas. One of the significant aspects of

oceanic plateaus (of which the Ontong Java is the largest, being one quarter of the size of Australia) concerns their possible role in the growth of continents⁶. Palaeomagnetic data will provide an important means of tracing their movements throughout past epochs, as all of the drill sites on the Ontong Java Plateau reached their present equatorial locations only relatively recently (during the late Neogene — the past 5 million years). Massive outpourings of basalt during the early Cretaceous, of the sort that now seem to have formed the Ontong Java, have been postulated before⁷. They would have caused changes in global sea level and — if occurring over a sufficiently short time interval — also in oceanic circulation and chemistry.

But the principal palaeoceanographic questions addressed during this leg concerned the response of the ocean — specifically the biogenic sedimentation therein — to changes in the variation of mean temperatures across the planet. It is known that during the Neogene, the temperature gradient between the equator and the poles increased as a result of

expanding snow and ice fields in polar regions. This trend resulted from thermal isolation of the Arctic Ocean and the Antarctic continent (through continental drift) and from overall sea-level regression. Regression is the consequence of mountain building, which thickens continental crust and deepens the ocean basins; it exposes land surfaces, which reflect more sunlight than the sea, and thus increases the planetary albedo.

The way that the global oceans responded to this increasing temperature gradient would have been mediated by the major changes in geography that were taking place at that time — the opening of the Drake Passage between South America and Antarctica, the intermittent build-up of the Antarctic ice cap, the closing of the Tethys seaway (which once connected the Atlantic and the Pacific from the Mediterranean region to the Himalayas), the rise of the Himalayan mountains and the separation of the Pacific from the Atlantic by the Isthmus of Panama. The combination of this evolving geographic setting and the increasing latitudinal temperature gradient would result in an episodic reorganization of established patterns of heat flow and ocean productivity, and thus in episodic changes in plankton distributions and in sedimentation of biogenic matter.

The Leg 130 sediment cores gave insight into these complex processes. The sedimentation-rate profiles for each site (Fig. 2a) show that the overall sedimentation rate for the past 25 million years is dominated by a low in the late early to early middle Miocene (20 to 15 Myr ago) and a prominent peak in the late Miocene to the earliest Pliocene. The crossing of the equator by some sites (indicated by squares in Fig. 2a) had no or little influence on these trends. At sites 803 and 804, the periods of low sedimentation rate are characterized by hiatuses (denoting levels where sediment is missing), suggesting sporadic sediment removal by erosion or mass movement⁸; but sites 805–807 seem to be biostratigraphically complete. The onset of the low-rate period coincides with a marked change in the carbon

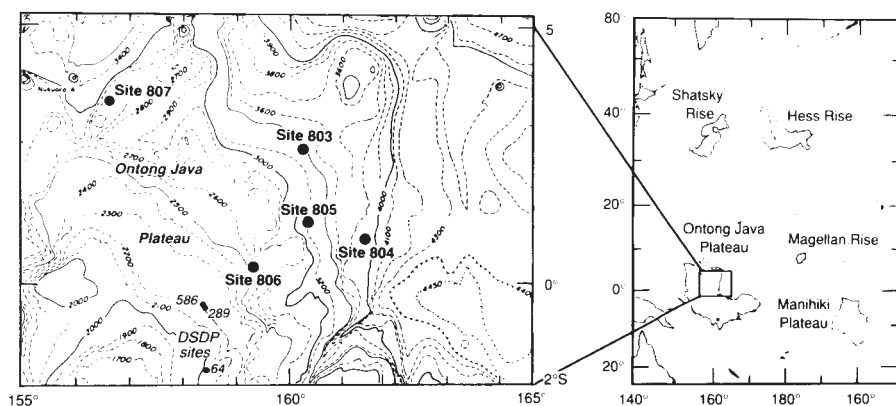


FIG. 1 Location map of Leg 130 sites. Bathymetry is in metres.

isotope composition of the ocean⁹, which is the result of an increased difference in productivity between the deep sea and coastal waters. Increased coastal productivity captures a greater share of nutrients

tion rate dropped at the sites drilled.

Since the middle Miocene, sedimentation rates at all the sites have been similar. The amounts of carbonate deposited (Fig. 2b) are also highly correlated between

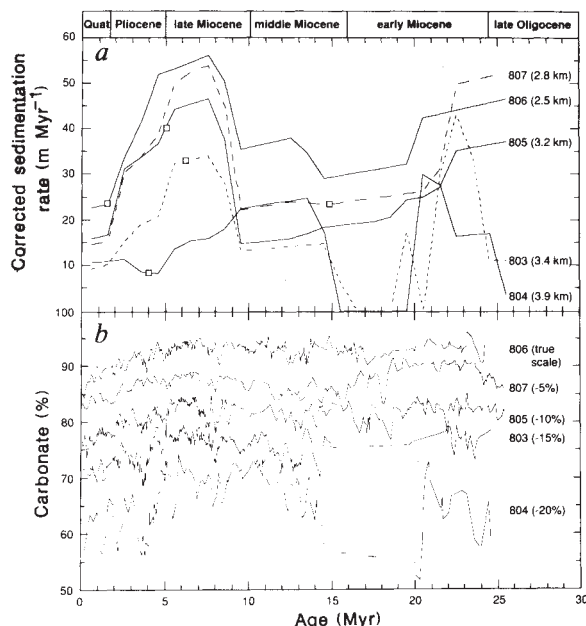


FIG. 2 a, Sedimentation rates (corrected for compaction) as a function of age for Leg 130 sites 803–807. Water depth for each site is indicated on the far right-hand side. Squares indicate the times when the sites crossed the equator. b, Carbonate percentages as a function of age for Leg 130 sites 803–807. Note that the absolute carbonate values for the sites have been adjusted by 5–20 per cent (see right-hand side of figure) to facilitate comparison. Acoustic reflectors in the seismic profiles often coincide with sharp changes in carbonate content.

for the margins, with the result that sedimentation in the deep sea is decreased.

The period of high sedimentation rate, centred around 7 Myr, in fact displays some of the highest rates (about 50 m Myr⁻¹) ever recorded in open-ocean pelagic sediments. The timing of this period suggests that these high rates may be associated with the closing of the Tethys and with important phases of mountain-building, leading to an increased supply to the ocean of continent-derived materials. At this time, a pronounced gradient of productivity developed from east to west in the equatorial Pacific^{10,11}. Eventually the piling up of warm water in the western Pacific, owing to increased trade winds, led to a reduction of productivity. The high sedimentation rates fell off during the Pliocene and especially during the Quaternary, accompanied by decreasing preservation of diatoms and radiolarians in the sediments. This drop also may be the result of removal of nutrients and silica along the coastal oceans by increased coastal upwelling. This finding is puzzling because we must assume that strong trade winds continued to drive upwelling in equatorial regions, and yet the sedimenta-

tion rate dropped at the sites drilled. Since the middle Miocene, sedimentation rates at all the sites have been similar. The amounts of carbonate deposited (Fig. 2b) are also highly correlated between sites for the past 12 million years, but poorly correlated before this. These observations suggest that a strong vertical gradient of carbonate saturation, which controls the increase of dissolution of carbonate with depth, became established at this time as a result of increased cold deep water formation. But it is puzzling that the carbonate values of deep sites are so similar to those of shallow sites. Carbonate removal by dissolution cannot by itself explain simultaneously the (distinct) differences in sedimentation-rate patterns between the sites, and the (slight) differences in carbonate content. More will be learned on this point by improving the resolution of sedimentation rates through analyses of Milankovitch frequencies in the record, of physical properties and of stable isotopes.

An important objective of Leg 130 was to account for the strong acoustic reflectors that give a 'layer-cake' appearance to seismic profiles of the sediments. Many of these reflectors correspond to the sharp changes in carbonate content seen in Fig. 2b, and there is some correlation between sites. On the whole, the results support the proposition that at least some of the major reflectors are caused by palaeoceanographic events (such as dissolution pulses) that affect carbonate deposition^{12,13}. □

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Animal warmth

ANIMAL fur and feathers are wonderfully self-adjusting. An animal or bird can fluff up its coat in cold weather for extra insulation, or smooth it down in the warm to dissipate heat. Daedalus is now imbuing human clothing with the same thermostatic cunning.

He is exploiting the 'bimetallic strip' principle: a laminate of two materials with different coefficients of expansion curls with change of temperature. Two-component synthetic fibres are already well known, so it should be easy to bond two polymers of contrasting expansion coefficients, like nylon and polyester, into a highly thermosensitive fibre. Inserted as flock or tufting in a conventionally woven fabric, such fibres will form a velvety 'Cat-fur'. Its pile will fluff up in the cold, but curl over and lay flat in the heat, neatly thermostating the wearer.

DREADCO's Cat-fur will come with a range of transition temperatures. For coats and other outerwear, it should fluff up at moderate outdoor-air temperatures, so as to respond to changes in the weather. A higher-temperature grade will be used in underwear. Responsive to typical skin temperatures, it will maintain intimate comfort in the face of wide swings of metabolic rate, from the hot sweat of exertion or anxiety to the chill of desperate foreboding.

The ultimate in thermostatted clothing will be a complete one-piece 'Cat-suit'. Each element of its surface will incorporate the right mix of Cat-fur fibres to maintain the skin beneath at its optimum temperature. It will be equally comfortable indoors or outdoors, in winter or summer, in violent exercise or total relaxation. The wearer will need no other garment!

An extreme test of Cat-fur will be at night, in its role as pyjamas or bed-clothes. A sleeper produces so little heat that he needs the maximum of thermal insulation; even so, modern beds (especially duvets) probably overdo it. A time-lapse film of a sleeper looks like a fight with an invisible boa constrictor, but is really a constant search for thermal equilibrium. When overheated, the sleeper must move to a cooler part of the bed, which soon heats up in its turn; when too chilly, he must curl up to conserve heat. This ceaseless unconscious activity is far from restful.

In blissful contrast, a sleeper insulated by DREADCO's Cat-fur will be in perfect thermal equilibrium all through the night. He will never have to move, and will truly 'sleep like a log'. A whole new era of total nocturnal comfort and perfect sleep will be ushered in! The dreary syndrome of daytime exhaustion, with its gummed eyelids and drained enthusiasm, will be abolished; instinctive zest for life, long-forgotten by the legions of Insomniacs Anonymous, will be restored.

David Jones

Link between scrapie and BSE?

SIR—Peter Aldhous recently reported¹ the view that transplacental passage of scrapie infectivity occurs in scrapie-infected ewes. But there are contradictory reports concerning this issue, reflecting our ignorance about the origins, spread and pathogenesis of natural scrapie. The elusive aetiology of scrapie underscores the difficulties encountered in designing and interpreting studies. As we describe here, some view natural scrapie as a viral-like illness whereas others describe it as a genetic disorder that happens to be experimentally transmissible.

Over the past 5 years, molecular biological and genetic data have profoundly altered our views of experimental scrapie in rodents and the trinity of human prion diseases — kuru, Creutzfeldt–Jakob disease (CJD) and Gerstmann–Sträussler syndrome (GSS). Indeed, the application of modern techniques to this enigmatic area of biology has begun to provide explanations about how a disease can be both genetic and experimentally or iatrogenically infectious^{2,3}. The main, and possibly only, component of the transmissible particle (or prion) in scrapie is an abnormal isoform of the host-encoded prion protein (PrP)⁴, which is generated by an as yet undefined post-translational process. That PrP has a central role in scrapie transmission and pathogenesis is independently supported by studies on the purification of scrapie prions, on the molecular genetics of scrapie incubation time genes and by analyses of infected neuroblastoma cells⁵.

Maternal (and lateral) contagious transmission of natural scrapie was first suggested by crosses of scrapied and scrapie-free Suffolk sheep. Progeny of affected ewes rather than rams were about seven times more likely to develop the disease⁶. In a subsequent study of Suffolks crossed with Scottish Blackface sheep a similar but reduced tendency was again apparent; ratios of approximately 1.9 and 1.3 to 1 were obtained in experiments involving 54 and 66 offspring, respectively. A 'background' scrapie incidence of up to 50 per cent in the progeny of unaffected parents, interpreted in terms of lateral contagious transmission, was apparent in this later study⁶. A tendency favouring maternal transmission in Parry's data⁷ on twins born of scrapie-infected ewes was noted by

Dickinson *et al.*⁵, although the numbers quoted for the progeny of unaffected ewes crossed with affected rams do not seem to correspond to the original data. But most of Parry's data on Suffolk sheep revealed no overt tendency for ewes rather than rams to transmit the disease⁷. Parry also found no evidence for contagious transmission — scrapie-free animals produced no affected offspring, even when crossed with scrapied animals.

The hypothesis of maternal transmission can be directly tested by bioassay for infectivity in scrapied ewes. Hadlow and coworkers failed to detect infectious titre in the uterus, ovary or mammary gland of clinically affected Suffolk ewes⁸. Two papers^{9,10} have been widely cited as indicating infectivity in the placentae of Swaledale ewes with scrapie. Unfortunately, negative controls for these experiments were not described and the incubation times in the inoculated recipients were scattered; an alternative explanation is that the observed instances of scrapie represent cross-contaminated inocula (but see ref. 15).

A few cases of transmission from experimentally inoculated ewes to offspring have been observed^{11,12}, but in an embryo transfer study by W. Foote *et al.* (personal communication) none of the offspring developed the disease within an observation period greater than or equal to 5 years ($n = 86$). Direct inoculation of control animals in these experiments produced a scrapie incidence of more than 51 per cent. Similarly, negative results were obtained for maternal transmission in experimental scrapie of goats¹³. Early reports suggesting maternal transmission of scrapie in mice have subsequently been strongly challenged. A maternal effect is not apparent in the transmission of either familial or experimental CJD, kuru or GSS.

In summary, the question of maternal transmission represents part of the larger issue of whether natural scrapie in sheep is a contagious disease under normal conditions of husbandry. As bovine spongiform encephalopathy (BSE) is inferred to have arisen by oral-dosing with contaminated feedstuff¹⁴, and given that some scrapie researchers considered that natural and experimental scrapie were not necessarily identical diseases^{7,15}, the data on natural scrapie may be irrelevant to the anti-

ipated behaviour of BSE. But as the dataset on experimental scrapie contains evidence both for and against 'maternal' transmission, and as there is a potential human health hazard concerning BSE, the resolution of this matter must come from future experimentation. Because our studies with transgenic mice¹⁶ show that the species barrier for passage of scrapie prions between rodent species is likely to reside in the amino-acid sequence of PrP, similar experiments seem relevant in examining the potential for transmission of prions from beef and sheep products to humans.

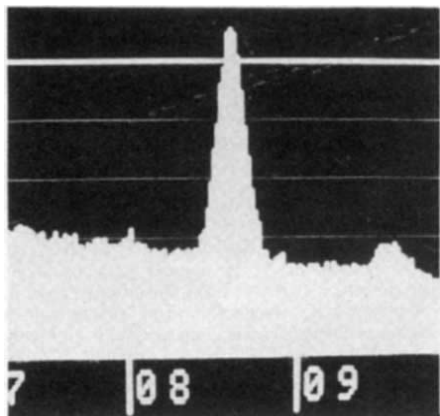
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Frustrated crystallographers

SIR—Dover's News and Views article¹, indicating that "many frustrated crystallographers are left wondering why their crystals will not grow large enough or well-ordered enough, or grew yesterday but will not grow today", brought back memories of my own experiences with this problem. When I first joined Professor Tom Blundell's group, good-sized crystals of the newly discovered avian pancreatic polypeptide hormone (aPP) had just been grown by Dr Steve Wood. But the small pot of crystals was soon depleted by low-resolution data collection and by screening more than 30 heavy-atom soaks by precession photography.

Subsequent laborious preparation of new materials and numerous crystallization trials would no longer result in crystals. Suspecting the presence of an unknown co-factor, I analysed the crystals using scanning electron microscopy. The microscope was fitted with an EDAX system which could potentially be used to identify trace elements by measuring their



EDAX spectrum from an aPP crystal indicating the presence of zinc.

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characteristic X-ray emission energies.

I placed one of the remaining small crystals of aPP on a sample stub to air dry and to my surprise it did not disintegrate. After coating it with carbon, I examined the sample under vacuum in the microscope and it remained crystalline in appearance. I recorded EDAX spectra over a 400-s time period and observed a peak at 8.63-KeV characteristic of the K_{α} line for zinc. The addition of zinc acetate to the crystallization experiments resulted in much larger crystals, indicating that the original scavenged zinc must have been limiting². The resulting high-resolution X-ray analysis indicated the key role this divalent ion played in forming

the crystal lattice^{2,3}.

I have also applied this technique to porcine 2-zinc insulin crystals and to freeze-dried iron containing transferrin. The ability to detect and identify traces of heavy elements acting as essential cofactors in protein crystals may provide one escape route for the baffled crystallographer.

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Expanding family

SIR—We have recently described¹ a family of six fungal genes involved in mitosis and the regulation of RNA synthesis whose protein products have in common a repetitive sequence motif of 34 amino acids (tetratricopeptide repeat or TPR). Zhang *et al.* (manuscript in preparation) subsequently found 16 tandem TPR sequences in the *crooked neck* (*crn*) gene involved in neurogenesis in the fruitfly *Drosophila*. We have now identified two additional family members in the yeast *Saccharomyces cerevisiae*.

The product of one gene, first cloned in 1983 (refs 2, 3), is a protein of relative molecular mass (M_r) 70,000 and was used for studying the targeting of mitochondrial proteins encoded in the nucleus. The 41 amino acids at the amino terminus

anchor the protein to the outer mitochondrial membrane and the rest lies in the cytoplasm. It is this portion that contains seven tandem TPR sequences; this arrangement of TPR repeats is remarkably similar to that of the cell-division cycle (CDC) subset of the TPR family¹. It is known that a truncated version of the gene, whose product is shortened at the carboxy terminus by 203 residues, including almost all of the tandem TPR domain, is anchored normally but produces cells with the same mutant phenotype as that of cells that lack the protein. Could it be that the repeated sequence domain mediates the interaction of mitochondria with the cytoskeleton much as *nuc2*⁺ is thought to interact with the nuclear 'scaffold'⁴?

The product of the second gene, *STII*, is a protein of M_r 66,000 induced by stress but not similar to the HSP70 family of heat-shock proteins⁵. Again there are internal TPR sequences, but of variable length, in an arrangement resembling that of the product of SKI3 gene involved in RNA regulation.

The inferred amphipathic helical structure of the TPR unit⁴ suggests several possible functions, including protein–lipid interaction and the mediation of protein dimerization. Our recent (unpublished) finding of intragenic complementation between temperature-sensitive alleles of *CDC23* by mutations in different TPRs suggests that protein–protein interactions are necessary for *CDC23* function.

In our original TPR dataset¹, we identified two more highly conserved subdomains, A and B, corresponding to the 'hole' and 'knob' in the repeat–repeat interaction model of *nuc2*⁺ function⁴. In the new examples, the B sub-

motif is maintained but there are different patterns of sequence conservation in A. We believe that TPR genes have evolved different functions based both on divergence of TPR sequences and also on the acquisition of separate, unrelated functional domains such as the targeting/anchoring sequence in the *M*, 70,000 outer mitochondrial membrane protein and the *zeste*-like domain in *SSN6* (ref. 1). Consistent with this idea, we have now found that single amino-acid substitutions in *CDC23*, which cause a temperature-sensitive phenotype, cluster into two separate domains: the carboxy-terminal TPR sequence block and an amino-terminal domain of unique, unrelated sequence.

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Proportional time

SIR—If there is unease with the idea of a defined moment for the Big Bang, with no temporal predecessor, why not adopt E. A. Milne's logarithmic proposal, where $\tau = t \log(t/t_0) + t_0$, with τ as dynamical time and t as conventional time (*Proc. R. Soc. A* **158**, 324–348; 1937)? As Milne remarks, the epoch of creation $t=0$ on the kinematic scale is measured by $\tau = -\infty$ on the dynamical scale.

Indeed, more generally there is a case for thinking of time in proportionate rather than in arithmetically progressive terms. In sensory physiology, strength of sensation is usually proportional to $\log(\text{stimulus})$; in pharmacology, drug effect is usually proportioned to $\log(\text{dose})$; and on a wider canvas, wage negotiations, inflation, growth and what-not, are almost always expressed in proportionate terms. Why should the philosophy of time restrict itself to an unnatural arithmetical scale?

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a		residues:
ALALDKKGNQFFRNKKYDDAIKYYNWALE	LKED	(99–131)
PVVFYSNLSACYSVSVGDLKKVVMSTKALE	LKPDY	(132–165)
VNSYIYLMALIMADRNDSTTEYYNYFDDK	LKDSNN	(363–396)
SSVYHRRGMNFILQNYDQAGKDFDKAKEL	LDPEN	(397–430)
PEVVPNFFAEILTDKNDFFDKALQYDLAIE	LENKL	(465–498)
LVGKATLLTNTPTVSENFTEATNLEKASK	LDPRS	(508–541)
EQAQIKGLAQMKLQQED	LDARTM	(542–575)

b		residues:
ADKEYKQGGNAFTAQDYDKAIELFTKAIEV	SETF	(5–38)
SKGYNRLGGAHLLGLDLDDEASNYKKKALE	LDAEN	(74–107)
ADKEKAEKGFYKARQFDEAIEHYNNKAWEL	LHKDI	(262–295)
SKSPARI GNAYHKLGLDLEKTEYYQKSLTE	HRFA	(336–369)
DILTKLRNNAEKELKKAEAEAYVVPFK	(370–395)	
ARGYSNRAAALALMSFPEAIAADCNKAIEK	DPNF	(430–463)
VRAVIRKATAQIAVADKEYASALETLDAA	(464–490)	
***	*****	
***	*****	
c		
A		B
AEAWFGLGHIYEKLGLDLEKALDAFQKALEL	DFNN	TPR
VRLWIKYARFEELLKEFLDRAREIYERALE	FLPRD	crn
PVAYIDLQAINFDVDEDEAIEKLEFDKALE	LDPDN	OMMP
AKGYAREGAALAKLGLDAEAIEDYKKALE	LDFAA	STII

Internally repetitive sequences and their statistical significance were identified and characterized by standard methods as described in ref. 1. The *M*, 70,000 protein and *STII* have intra-sequence comparison scores of 7.09 and 12.37 s.d. units, respectively; inter-sequence comparisons with other members of the TPR family yielded scores ranging from 3.44 to 12.43 s.d. units. Only the repeats of highest significance are shown. Starred columns, the most highly conserved positions in the aligned sequences. The original TPR motif was based on 47 repeats from five different proteins and showed two more highly conserved subdomains labelled A and B and represented by the sequences W...LG...Y and A...(Y/F)...A...P, respectively (ref. 1). The CDC subset of TPR proteins includes *CDC23*, *CDC16* (*S. cerevisiae*), *nuc2*⁺ (*S. pombe*) and *bimA* (*A. nidulans*); the RNA regulation subset includes *SKI3* and *SSN6* (*S. cerevisiae*). To facilitate comparisons with new candidate TPR genes, a file containing all known TPR protein sequences, as well as suggestions for quantitative comparisons, is available from M.S.B.

Historical light on photography

SIR— During the historical session of the French Academy of Sciences on 19 August 1839, F. Arago disclosed the now well-known daguerrotype process invented by Daguerre¹. But a few years earlier, in 1816, Joseph Nicéphore Niépce obtained the first negative picture on a paper coated with silver salts and placed in a crude camera obscura². The picture was sent in a letter to his brother, but it was not fixed, and he also failed to produce a positive from it.

Niépce investigated numerous light-sensitive compounds which he classified into three types with respect to the effect of light: "colouring properties, discolouring properties and solidifying properties". He eventually succeeded in irreversibly fixing on a support the reflection of light from a landscape by using asphalt spread on glass, stone or metal. In 1829 he described his process which was published 10 years later³. Niépce had discovered that bitumen hardens on exposure to light, thus making it possible to capture the imprint of light and shadow of an image by removal of the unexposed areas with an appropriate solvent.

But the process, termed heliography by Niépce, remained obscure. Only a unique picture called *Point de vue du Gras*, obtained by Niépce in 1826, is known and his work cannot easily be evaluated from this single heliograph.

We have attempted to reproduce Niépce's technique and in June 1989 were able to produce the first heliograph ever made since Niépce's time⁴. We followed all his written methods, and experimentally determined the exposure time, which he never mentioned. The heliographic process is, in practice, rather different from the interpretation given by historians and provides a clearer understanding of some of the explanations given by Niépce in his letters.

The photosensitive compound used by Niépce was bitumen of Judea, or asphalt⁵. In Niépce's process, bitumen is dissolved in oil of lavender and spread as a thin layer on a well polished silver-coated plate as a varnish. This constitutes a photosensitive film which, under the action of light, partially loses its properties of solubility due to a crosslinking process⁶. This variation of solubility with dose allows one to dissolve the areas where the varnish remains soluble — those which have not been illuminated — while it becomes insoluble in the exposed areas.

The bitumen we used is a commercial product (Prolabo) in the form of a brown powder. Our preparation of the well-polished silver plate, covered with the bitumen varnish to obtain a thin golden brown film follows exactly the operations described by Niépce¹. The optical absorption spectrum of a thin film of bitumen

prepared from a bitumen solution (3 g in 10 ml of lavender oil) shows an optical density at wavelength's under 500 nm.

In the literature of the history of photography, the formation of an image by the Niépce process is explained as the simultaneous hardening and whitening of the bitumen under the action of light and thus would be a direct positive process^{2,6-8}. Our first experiments were performed by irradiating homogeneously the light-sensitive plate either with sunlight or with a



Top: negative image obtained with bitumen of Judea by using contact printing (see text). Light areas correspond to bright silver, dark areas consist of crosslinked brown bitumen. Bottom: direct reaction of iodine vapours on the negative image leads to the formation of silver iodine only where the bright silver of the negative image was apparent. Under the action of light these bright areas turn black. After complete removal of the bitumen still present, the positive image is obtained. (*Le Gras*: Niépce's house at St Loup de Varennes near Chalon-sur-Saône, France).

halogen lamp whose light intensity was controlled; the bitumen hardened under these conditions. But no change in the golden brown colour was detected even after a long irradiation with visible or ultraviolet light. Niépce said that no colour variation is observable after exposure to light. There is no whitening of the bitumen with light, contrary to what has frequently been claimed^{1,2,6-9}.

Niépce's method of obtaining a positive image is the following: the unprotected bright silver is blackened by submitting the negative plate to iodine vapours. This leads to the formation of silver iodine at the surface of the plate which is then transformed, under daylight, into finely divided black silver. The last operation consists of stripping the hardened bitumen off the plate with alcohol. In its final form, no trace of bitumen is left on a heliograph. The bitumen plays the role of an inter-

mediate compound, like a negative image laid on silver. Iodine vapours penetrate the different areas of bitumen, proportional to their density, and restore all the variations of greys from black to white. The shadows are made of black colloidal silver, while the lights are polished silver. The image we obtain has a high resolution (80 lines per mm) and good reproduction of shades of grey.

Our second important result concerns the exposure time. Many hypothesis have been suggested⁷ as Niépce never clearly mentioned this parameter. Three different methods have been used to obtain heliographs: (1) by projecting a slide magnified from 24 × 36 mm to 80 × 100 mm with a slide projector equipped with a 150-W lamp and a *f* 2.8-aperture lens (about 55,000 lux on the bitumen). The exposure time ranges from 10 to 15 hours. (2) By contact printing under the Sun (about 350,000 lux) of a positive modern photograph on a transparent film. The exposure time varied from 1.5 to 3 hours. (3) In a camera with a *f* 3.5 aperture lens (similar to Niépce's method for obtaining heliographs with a camera obscura). In this case, our experiments showed that the level of light on the plate was 5,500–11,000 lux. Therefore an exposure time 60–100 hours is required. These times are in agreement with those mentioned by Biot in 1839, who wrote that the Niépce process needed 2 or 3 days under a very intense light to obtain an image from nature in a black box¹⁰. Thus Niépce could only heliograph static subjects in very intense light. But Niépce's discovery opened the way to his successors.

The exceedingly low sensitivity of bitumen of Judea explains why the different steps of the whole process, such as the preparation of the varnish or its dissolution after exposure, can be carried out under room light without damage. It seems that the determination of the exposure time is the main obstacle preventing the reproduction of Niépce's process. Some historians have erroneously concluded that the exposure time was 8 hours for a landscape illuminated by a bright Sun⁹.

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Stripes on a pterosaur wing

SIR—D. Martill and D. Unwin in their recent Letter to *Nature*¹ described a pterodactyl fossil in which details of the soft tissues of the wing membrane were preserved. Scanning electron microscopy revealed an epidermal layer on one surface of the specimen, with coarse ridges and finer furrows, below which was a spongy layer said to contain blood vessels. Martill and Unwin interpreted this 'stratum vasculosum', together with an underlying coarsely crystalline layer, as the dermis. Below that was a layer they described as striated muscle, which apparently formed the middle of the original membrane, but the authors did not explain why they thought this layer was muscle. It consisted of polygonal prisms with a maximum diameter of about 40 µm, on which Martill and Unwin said that "transverse striae and nuclei" were visible at high magnification. They did not illustrate these striae, or give any details. The features seen in the electron micrographs would presumably be annular ridges or grooves around the circumference of the prisms.

There are examples of fossils in which very fine details of the surface of soft tissues have been preserved, apparently by the deposition of a calcite 'mould' of the surface, before the tissues decayed^{2,3}. This process preserves only surface relief, and it is unlikely that intracellular structures such as muscle striae (sarcomeres) could be preserved in this way. The sarcomeres of fresh striated muscles have a solid appearance under the light microscope, but are not accompanied by annular corrugations on the surface of the fibre. It is conceivable that the sarcolemma might be deformed into annular wrinkles in a strongly contracted muscle fibre, but striae formed in this way would not represent sarcomeres. For a surface mould to be formed that would show such features, the muscle fibres would in any case have to remain in tetanic contraction for some time after death, which seems unlikely. On the other hand, passive elastic fibres would contract when the animal died. Elastic fibres, if they were enclosed in a relatively inextensible sheath, might well exhibit a surface pattern like that reported by Martill and Unwin in their paper.

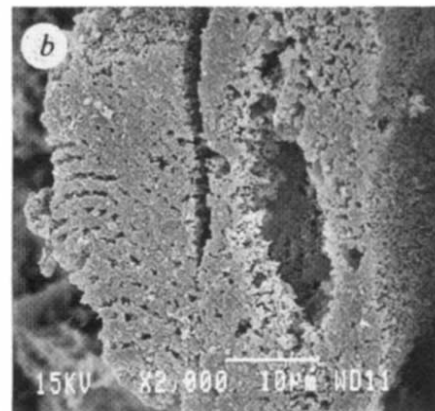
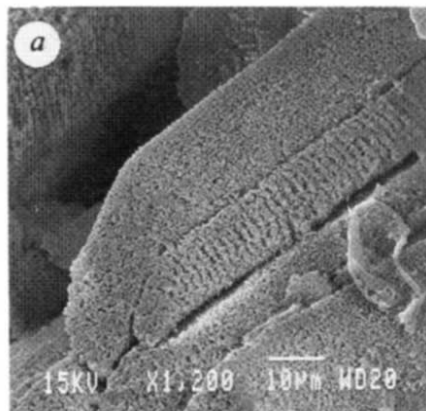
I reviewed evidence² indicating that the ridges on the wing surfaces of certain pterosaur fossils are not stiffening fibres, as had been previously thought, but are wrinkles in the surface of a soft membrane, caused by the contraction of robust (but unseen) internal elastic fibres. These hypothetical elastic fibres would have to be orientated transversely to the wrinkles, not parallel to them as shown by Martill and Unwin in their Fig. 3c. Martill and Unwin's observations could be inter-

preted as the first direct evidence for the existence of these fibres.

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MARTILL ET AL. REPLY—Pennyquick suggests that we may have misinterpreted the polygonal fibres in our exceptionally well-preserved pterosaur wing membrane from the Santana Formation (Lower



a, Striated muscle fibres from pterosaur wing membrane from the Santana Formation, Brazil. b, Oblique section through individual fibre demonstrating continuity of surface banding through body of fibre. We interpret the banding as fossil sarcomeres.

Cretaceous) of Brazil. We concluded that the fibres represent striated muscle replaced by cryptocrystalline calcium phosphate. Pennyquick believes that the fibres represent his hypothetical 'elastic' fibres², which he suggested as a possible mechanism for contraction of the flight membrane on the basis of impressions in fine-grained sediments. We here review our evidence for interpreting these fibres as striated muscle and report briefly on our new studies of this tissue.

The most common soft tissues found in the Santana Formation are striated muscle and gill lamellae of elopomorph fish⁴. Here, the muscle fibres are found in their original myomeres, and there is no doubt that they represent fossil striated muscle fibres. They even show sarcolemmic membranes, cell nuclei, myoseptal membranes and sarcomeres⁵. Preservation is by replacement with calcium phosphate as demonstrated by powder X ray diffractometry. The fibres we found¹ resemble very closely the muscle fibres found in the fish. Furthermore, pterosaur fibres display a regular banding seen on the surface.

Pennyquick suggests that striated muscle would not be preserved as a series of ridges on the surface of a fibre. Indeed in this respect he is correct. Our striated muscle fibres are not preserved as a series of ridges. Instead, they are preserved as a series of stacked 'disks' along the length of each fibre (a in the figure). We have carefully made a new section through the

preserved muscle fibres such that several individual fibres have been cut obliquely, showing that the disks are present through the fibre, as would be expected if sarcomeres were preserved (b in the figure).

Pennyquick's surprise at the exceptional preservation of our material could be because the material he examined was represented by external moulds in fine-grained micrite. This style of preservation can only reveal details of external surfaces, the resolution of which is governed by the grain size of the sediment host. Our material, on the other hand, is a replace-

ment, in three dimensions, by crystallites with an average length of 300 nanometres. The resolution achieved by this style of preservation allows the identification and accurate observation of three-dimensional intracellular structures. We are confident that the fibres are those of striated muscle tissue.

But our new section shows some structures between the muscle fibres and the stratum spongiosum (labelled sparry calcite and kerogen in Fig. 3a of ref. 1), as yet unidentified, that could be Pennyquick's hypothetical elastic fibres or Wellnhofer's stiffening rods⁶. In either case it appears that the pterosaur wing membrane is a highly complex organ, not just a piece of stiffened skin.

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■ Martill's data on fish, referred to above, are published on page 171.

Matters of life and death

F. E. G. Cox

Lives at Risk: Public Health in Nineteenth-Century Egypt. By LaVerne Kuhnke. University of California Press: 1990. Pp. 233. \$40.

NINETEENTH-century Egypt was not an idyllic place. Dysentery, cholera and Egyptian chlorosis were endemic in the villages and sewage often contaminated the water supplies of Alexandria and Cairo. Malnutrition was everywhere and epidemics of smallpox and plague had ravaged the whole country by the turn of the century. The medical expertise that did exist was largely based on mediaeval Arab medicine but practised, not by professionals of earlier centuries, but by hakims whose training consisted of copying the standard texts in order to memorize them. It is against this background that LaVerne Kuhnke traces the development of public health care in Egypt, with numerous side glances at the implications for health in the developing countries today.

Changes in Egypt began when Muhammad Ali, who had seized power as governor in 1805, formed a conscript army and, anxious about its health, introduced Western medical institutions and technologies which, Kuhnke argues, were not always appropriate to the needs of the

country. In 1827, Ali established a Western medical school modelled on those in France and staffed largely by Italians. Twelve of the first batch of graduates continued their training in Paris in 1832 and five returned to form the nucleus of the school. Their successors made significant contributions to medicine, the most notable being Griesenger, who found that Egyptian chlorosis was caused by hookworms, and Theodor Bilharz, who discovered *Schistosoma haematobium*, the causative agent of the disease that now bears his name, bilharzia. In 1832, a medical school for the training of hakimas (women licensed to practice medicine) was founded, a remarkable achievement in a Muslim society. By 1840, responsibility for health care had also passed to headmasters in schools, with severe penalties for negligence. Thus, by the middle of the century, the elements of public health care had been well established and epidemics were no longer allowed to run unabated.

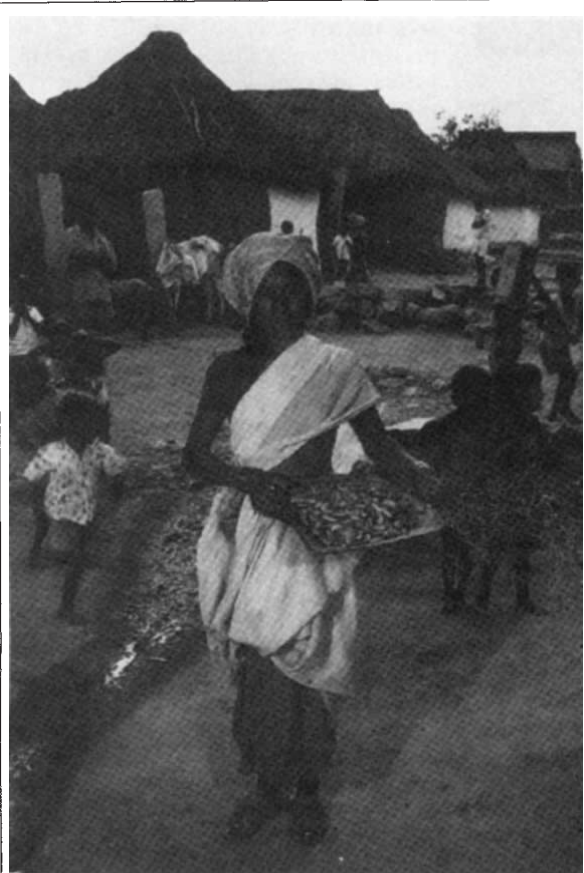
Epidemics of cholera in 1831 and plague in 1834–36 were countered by rigorous

quarantine regimes. An international quarantine board had been set up some years earlier on advice given by the French council of health, but the 1831 cholera epidemic caught it unprepared. By 1848, as a result of a combination of quarantine measures and the implementation of British sanitary reforms, the next epidemic, although severe, was more easily contained. Plague also ceased to be a serious problem and after 1844 it disappeared from Egypt for 55 years. Vaccination against smallpox was both efficient and effective and between 1837 and 1840, 61,000 children had been vaccinated in Cairo alone although there was strong opposition and non-compliance in many villages.

The evidence presented seems to suggest that the control schemes used actually worked, but Kuhnke questions whether the Western ideas on which they were based were appropriate for Egypt. This is the weakest part of the book and detracts from an otherwise scholarly and dispassionate work. The author perceives an apparent distinction between "authentic medicine", equated with curative medicine, and community and public health concerns, concluding with these words: "As long as the West upholds urban hospital-based curative medicine for the individual as the ideal for 'health care', the lives of rural Egyptians and many others may continue at risk." This is an entirely false picture of how biomedical science interacts with the needs of the developing countries (see, for example, *Biomedical Science and the Third World*, edited by Barry Bloom and Tony Cerami, *Annals of the New York Academy of Sciences*, Vol. 569, 1989). Tropical medicine, and the World Health Organization in particular, is as much concerned with prevention as with cure and the two run together. It is a pity that Kuhnke did not consider bilharzia, as this is a disease that has always been associated with Egypt and from which much can be learned. The conflict for public health strategy is between the needs of water for irrigation and the consequent inevitable spread of the snail vectors. Control of bilharzia is based both on snail control and on chemotherapy, which is essential to reduce contamination of snail-infested waters.

History does repeat itself, and the resistance of some villagers to vaccination and their failure to return for booster doses is still a problem today. It is also interesting that seven of the first twelve graduates of the medical school chose to remain in Paris, and the pile of examination scripts now on my desk reminds me that twentieth-century students also learn large parts of textbooks by heart. □

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More than half of India's land surface is cropland — the soils are fertile and robust, and rainfall adequate. These factors have combined to make India the most densely populated large nation on Earth; 80 per cent of its inhabitants live directly from the land or in the service of small rural communities. From *Man on Earth*, just published in Penguin paperback, by John Reader. First published by Collins in 1988, the volume records the author's attempts to understand humanity. Reader looks at culture from a functional point of view, looking at how adaptation and regulation have promoted the continuing existence of people in a wide variety of environments throughout the world. Illustrated with the author's own superb photographs, price is £9.99.

In the balance

Edward Abraham

Miracle Cure. The Story of Antibiotics. By Milton Wainwright. Blackwell: 1990. Pp.196. £16.95.

MILTON Wainwright has written a readable pot-pourri. Much of it is concerned with the assessment of credit for the discovery of the clinical value of penicillin and streptomycin, but the author strays into other areas, including a description of some bizarre procedures that have previously been claimed to cure bacterial infections and the fiasco of the antibiotic patulin and the common cold.

The author, who is in the Microbiology Department at the University of Sheffield, states that he has "attempted to redress recent attempts to downgrade the role played by Sir Alexander Fleming in the discovery of this most famous antibiotic". Some may wonder whether this is necessary, for Fleming's name has been embedded in the public mind, while Florey's and Chain's have not. Yet Fleming was not elected to the fellowship of the Royal Society until 1943, 20 years after he had first been proposed by Almroth Wright. Wainwright suggests that this should carry little weight with "anyone who is familiar with the routes by which one can succeed to this august body". The precise implication of his phrase is not clear. But great trouble was taken to ensure that Fleming's candidature was not adversely affected by publicity for the findings in Oxford in 1940 and 1941. And it should be remembered that the Royal Society is concerned with science, not merely with medicine.

Fleming's chance discovery of penicillin has never been seriously disputed, nor has his early belief that it might be clinically useful for local application to infected wounds. The vital discovery at Oxford, which receives little emphasis in the book, was that even exceedingly impure penicillin could cure generalized and life-threatening infections when introduced into the blood stream. It was this that gave penicillin its outstanding medical importance. All that is arguable is whether Fleming could have demonstrated this property in mice if he had thought of trying to do so.

This book contains several interesting anecdotes which may not be widely known. Appropriately, it describes in some detail the striking success in Sheffield of Dr C. G. Paine, whose penicillin-containing culture filtrates from Fleming's *Penicillium* were used for local treatment of eye infections in about 1930. Paine never published these results, but later he sent notes on the cases to Florey. These notes (not mentioned by Wainwright)

were reproduced in 1949 in *Antibiotics* by Florey *et al.*

Wainwright provides simplistic and slightly misleading accounts of the chemistry of penicillin and the semi-synthetic penicillins and cephalosporins. Because all these compounds have a four-membered β -lactam ring it might have been more appropriate to have placed them in the same section of the book.

The discovery of streptomycin was reported in 1944 by Schatz, Bugie and Waksman at Rutgers University; this antibiotic was later found to be effective in the treatment of tuberculosis. This book describes how all was well between Waksman and Schatz until Schatz discovered that Waksman was receiving

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Fleming — "embedded in the public mind".

money from Merck and began a lawsuit for a share of royalties. The case ended with a partial victory for Schatz. However, when a Nobel prize was awarded to Waksman alone, Schatz could not bring himself to remain inactive but, predictably, he gained nothing from his activity. Wainwright states that he has tried to redress an historical imbalance, "this time in favour of Albert Schatz". Whether the balance needed redress is at least open to question. Perhaps Waksman was ungenerous to Schatz and too secretive. But it seems that Schatz failed to appreciate that he was a research assistant to a man who had opened a wide field of investigation that had already led to the discovery of streptomycin. What is beyond doubt, however, is that misjudgements by Waksman and Schatz brought them both a great deal of misery.

This book is well produced. But some readers would find a more detailed index more useful and others might appreciate the addition of a few primary sources to the author's suggestions for further reading — to redress its balance. □

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A way through the jungle

Colin Gough

Physics of High- T_c Superconductors. By J. C. Phillips. Academic: 1990. Pp.393. \$55, £36.

ALMOST four years after Bednorz and Muller's Nobel prizewinning discovery of superconductivity in the layered cuprate compounds, the unexpectedly high transition temperatures of these materials remains unexplained. It is frequently claimed, with some justification, that there are as many theories for the layered cuprate high-temperature (or HTC) superconductivity as there are groups working on the problem. Keeping abreast of the literature remains a daunting task for even the most experienced researcher.

A book designed to summarize and explain the physics of HTC superconductors is therefore specially welcome, particularly when the writer is J. C. Phillips, who has made many significant contributions to the theory of the electronic and phonon properties of solids including conventional superconductors.

In aiming "to cut a well-marked path [through a literature] as forbidding as the darkest jungle", Phillips has selected a rather special path, carefully chosen to support his view that HTC superconductors are really no different in kind from previously known superconductors. He is sufficiently confident to state "there is no room for doubt that it is lattice instabilities and not magnetism that produce high- T_c superconductivity just as much in cuprates as in intermetallic compounds". Relatively little attention is drawn to other dangerous animals lurking in the jungle in the guise of theoreticians with diametrically opposed views.

The original Bardeen-Cooper-Schrieffer (BCS) model successfully accounted for superconductivity in terms of an indirect attractive electron interaction leading to superconducting pairing via lattice vibrations. This mechanism appears to place an upper limit for superconductivity of around 40 K (-233°C) — well below the current record for HTC superconductors of 125 K (-148°C). However, Phillips emphasizes that the layered cuprate HTC superconductors are much more complex structures than the theory was originally developed to describe. Matthias, who spent a lifetime in the quest for higher temperature superconductors, would have claimed that the cuprate superconductors were "tricking nature" into circumventing the apparent boundaries of conventional BCS theory.

To support this thesis, Phillips first presents an extensive background to electron-

lattice interactions in old-style superconductors including simple elemental and binary alloy superconductors, binary and ternary compounds, such as the A15 and Chevrel phases. The superconducting cuprates are ternary, quaternary and even more complex chemical compounds — very much more complicated chemical structures than those usually studied by solid-state physicists.

Phillips describes the most important HTC compounds known up to about a year ago and argues the case for superconductivity via low-frequency lattice vibrations or instabilities. Two of the most carefully studied HTC superconductors are $(\text{La,Sr})_2\text{CuO}_x$ and $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$, in which the superconducting properties are controlled by strontium and oxygen vacancy dopings, respectively. The relatively facile diffusion of oxygen in the $\text{YBa}_2\text{Cu}_3\text{O}_7$ structure is related to soft lattice correlations involving oxygen vacancies — one of 'nature's tricks' to give superconductivity at 92 K for this compound.

Any successful theory must first account for the anomalous normal state properties. Others have claimed that the remarkably linear dependence of resistivity on temperature common to all the layered HTC compounds reflects an intrinsic property of the electrons in the layered cuprates. Phillips presents a more mundane explanation in terms of electron scattering by the same low-frequency lattice vibrations that ultimately lead to superconductivity.

Who is right remains an open question. On balance, more recent measurements would tend to favour the former view. But it would not surprise me if Phillips turns out to have the last word. The ability of theoretical physicists to adapt their theories to fit inconvenient experimental facts is legendary. Experimentalists can also all too easily become locked into believing the current fashionable theory, with no better justification than other competing but less fashionable theories. At this stage in the development of our understanding of HTC superconductors it would be wise to keep an open mind.

Provided the reader recognizes that this book is not an introductory text but is a research monograph intended to argue a particular point of view, it can be strongly recommended — particularly to those with a solid-state physics background. □

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■ Recently published by Plenum is *Oxygen Disorder Effects in High T_c Superconductors*. Edited by J. L. Morán-López and I. K. Schuller, the volume contains the proceedings of the conference held at the International Centre for Theoretical Physics in Trieste in April 1989. Price is \$59.50. □

Object lesson

Richard Davenport-Hines

The Science of Woman: Gynaecology and Gender in England 1800–1929. By Ornella Moscucci. Cambridge University Press: 1990. Pp. 278. £35, \$49.50.

THE history of childbearing is the subject of several recent and excellent books. In addition to Renate Blumenfeld-Kosinski's study of mediaeval caesarean births entitled *Not of Women Born* (Cornell University Press, 1990), there is Valerie Fildes' *Wet Nursing* (Blackwell, 1988) which traces changing attitudes to breast-feeding from antiquity to the present day, and *The Politics of Maternity Care* (Oxford University Press, 1990), in which Jo Garcia and her colleagues have collected a range of historical essays on services for child-bearing women in twentieth-century Britain. This burgeoning literature is now joined by Ornella Moscucci's study of the influence of gynaecological ideas and practice on scientific notions of gender.

Moscucci focuses on London teaching hospitals, but the implications of her study are wider. She questions science's autonomy from society, and challenges the neutrality of its mediations, arguing that the social relations between men and women in the nineteenth and twentieth centuries were reflected in medical notions about masculinity and femininity. She shows deftly and authoritatively how the personal and professional circumstances of British gynaecologists fashioned both the ruling principles of gynaecology and affected the treatment

that was offered. She analyses how the wish for professional exclusiveness turned gynaecological practices into weapons in the battle for professional hegemony — for example, how the "envy, hatred and malice" which existed between surgeons and obstetric physicians, especially in the 1884–1929, was fired by controversy over the issue of ovariectomies. The ill-effects on patients when hospital hierarchies become too ruthless in their self-perpetuation makes sad reading.

The scientific study of femininity in nineteenth-century western Europe began as a genre combining scientific findings with literary quotations and philosophical speculation. It fastened onto the processes of puberty, menstruation, childbirth and menopause as indications of an essential animality in women, with the result that medical writings increasingly depicted women as unstable, illogical and the victims of their own bodily emergencies. The subordination of women was enforced by medical teachings, while female sexuality was represented as a threat. An article in the *Obstetrical Journal of Great Britain* in 1874 typically described women as the "reproductive servants of their race", and a decade later a leading obstetrical textbook depicted the ovary as "ever struggling for supremacy, and cannot long be kept in subjection". The physiology and pathology of the female sexual system were represented as defining women's personalities. As a physician wrote in 1916, "it is because of all the internal secretions that woman is what she is".

Moscucci is far from representing all women as the passive recipients of male medical care, and shows how many people

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Thomas Spencer Wells, the British gynaecologist who perfected ovariectomy.

resisted inappropriate treatment by men. Her book offers many interesting side-lights on issues of female surgery, on the practice of antisepsis, on the use or abuse of the speculum, and on craniotomy as a technique in childbirth. She recognizes the complexities of her subject, and neither adulates physicians as infallible scientific titans nor lapses into the crude petulance of other medical historians who tend to present all patients as the victims of despotic medical authority.

This monograph is the latest volume in the Cambridge History of Medicine series, which is reliably distinguished by scrupulous research and fine historical judgement. (Moscucci is writing the anniversary history of Britain's Royal College of Obstetricians and Gynaecologists.) *The Science of Woman* maintains the quality of the Cambridge series, and although it has too much of the tautness of a PhD dissertation, it is imaginative and well-written. Moscucci's insights have been enriched by her reading in modern feminist historiography, but she dissents from some of its orthodoxies in passages which are notably reflective and honourable. The integrity of her ideas and the honesty of her doubts are as impressive as the range and power of her research. □

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Wearing sheep's clothing

Peter Coles

Trois Pattes pour un Canard (Souvenirs d'un biologiste). By Etienne Wolff. Editions de la Fondation Singer-Polignac, Paris: 1990. Pp. 201. FF130.

A CELEBRATED experimental embryologist, former director of the respected Collège de France and member of the Académie Française, Etienne Wolff has been nicknamed "the monster man". Although he dislikes — and rather resents — the title, it was inevitable. His most well-known work has been to produce live monsters in the laboratory — cyclopean chicks, a duck with a third leg where its tail should be, birds with heads sprouting from their intestines and others, equally grotesque. Commonplace in contemporary embryology, teratology was an unfashionable and little explored domain in the 1920s. Now, at the age of 86, Wolff has written a set of 'biographical essays' and a short note on his research to allow us a peek behind the off-putting nickname.

The image Wolff gives us of himself is sometimes candid, but the 'essays' remain mostly rather superficial and arbitrary. The overall theme is autobiographical, but the essays do not fall into chronological order.

Sandwiched in between "childhood" and "orientation of my studies and career", for example, is a chapter on "Judaism and anti-semitism". After a lifeless and rather banal description of his trips to Japan, the United States and the Soviet Union, there is a chapter on "animals and me", where he defends animal experimentation, saying that real cruelty is found in the commercial exploitation of animals, not in the laboratory. But accusations of cruelty associated with his research obviously weigh heavily, despite his light-hearted self-mockery, since almost too much space is given to show he is an animal lover. While the front cover shows a disturbing picture of a three-legged duck, the back cover has a picture of the author cuddling his own cat, "Grouillot".

At the beginning we are treated to anecdotes about Wolff's childhood and his early education, including descriptions of a Paris with steam trams and open-topped buses. Yet these do not really come alive, partly because the manuscript does not appear to have been edited, leaving a cumbersome and repetitive prose. Then there is a sketch of the meandering university studies that led from a degree in letters to a research post — rare at the time — at the Institute of Embryology of the University of Strasbourg. Here began his

initiation to embryology and the encouragement to explore the largely uncharted area of experimental teratology.

Early in his career, Wolff made some remarkable discoveries. He found that sex hormones, not proteins, determine the sex of chicks and that gender can be changed in mid-course of development. He also found that very specific damage to an embryo at a given date in histogenesis consistently produces a given 'monstrosity'. With the outbreak of war Wolff's career was suddenly interrupted and in 1940 he was taken prisoner. He spent the next five years in prisoner-of-war camps in Austria and Germany where, with a handful of fellow academics, he set up a 'mini-university', giving lectures to other soldiers. His two books, "Sex Changes" and "The Science of Monsters", published after the war, were both written in a camp in Germany. Returning to Strasbourg after the liberation, he built up a thriving laboratory with a staff of 50. This doubled when he was awarded a chair at the Collège de France in Paris, where his laboratory in the Bois de Vincennes became known as the Mecca of Embryology.

Wolff says very little about his research in this book. In a mere 50 pages out of the 200 he describes how he could produce, regularly and systematically, 'single' and 'double' monsters, cyclops and omphalocephalic chicks (with heads in their breasts). Other work included growing organ cultures *in vitro* and changing the sex of chicks. But although he is keen to dispel the idea that he is a kind of "Dr Frankenstein" — most of his monsters appear spontaneously in nature — we are not told why this work is important.

Much space is devoted to eulogies to Wolff's teachers, to friends made in the prisoner-of-war camps, and to his colleagues, yet even these sometimes lack depth. Similarly, for a Jew who found himself in the hands of the Nazis — and who also experienced the antisemitism of Pétainist France — Wolff's chapter on antisemitism is surprisingly incoherent and even comes across as an apology. He resents the word 'Jew', preferring to be called an Israelite — ironically one of the terms used by Arab antisemites.

All in all, this book will please Wolff's friends, colleagues and fellow academicians. But it does little to "sell" embryology to a broader public and gives a disappointingly reluctant, or over-modest, account of the life of one of the leading twentieth-century French biologists. One cannot help but come away with the impression of a brilliant, but very sensitive man who, while having enjoyed the highest accolade from his peers, has never come to terms with the abuse from his detractors. □

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The resurgence of science in France

The Socialist government of France has indeed done wonders for the health and achievement of French research, but there is a raft of problems — some of them long-standing — still to be resolved.

FRANCE, once the most conservative country in Europe, has become a land of technology buffs. People reserve seats on the high-speed trains whizzing everywhere (as well as theatre tickets) by Minitel, the domestic video-text machines that have swept the country — and which could not have functioned if the telephone system had not been superbly modernized in just ten years. France has also provided the impetus for the creation of the only civil aircraft manufacturer outside the United States capable of selling civil aircraft to the world's airlines. Now it is seeking to drag its European partners into space. What has brought about this transformation? Do its roots lie in the election of François Mitterrand's Socialist government in 1981, and in its deliberate and daring programme to reinvigorate science and technology?

Neither the nature of the transformation nor the importance of its apparent cause should be exaggerated. France remains the Western European state most apt (and able) to claim to be the custodian of European civilization. (The cuisine is merely a by-product.) And technology has not ruined the delectable appearance of France, only some of it. (Too many city mayors have commemorated themselves with ungainly blocks of concrete in mediaeval settings.) Civilization does not, of course, equate with civility, but with respect for the law (including the Revolution's slogan), for intellectual discourse and the importance of the imagination.

The impetus for change in the 1980s is also a prolongation of earlier tendencies. How could it be otherwise when even provincial street names commemorate Descartes, d'Alambert, Pasteur, the Curies (both of them) and the like? The modernization of French science and technology began with the Gaullist government of 1958 and with the creation of the Délégué Général de la Recherche Scientifique et Technique (DGRST), which rapidly transformed itself into a kind of unestablished ministry of science.

Much of the Gaullist government's support for science and technology sprang from its preoccupation with the military defence of France, but that is when modernization began in earnest. The decision in 1981 that the budget for civil research should increase by more than 16 per cent a year was but the arithmetical consequence of the decision that civil research and development should be 2.5 per cent of Gross Domestic Product by 1985. The name to conjure with is that of Jean-Pierre Chevènement, Mitterrand's first minister of research and industry, now defence minister in Mitterrand's second Socialist government.

The timing of this survey of French science is deliber-

ate: it may not be too soon to estimate the outcome of what an earlier survey (*Nature* **296**, 285; 1982) called "the grand experiment". The outcome should be of interest not only in France but elsewhere, in Britain for example, where governments have taken a more jaundiced view of the benefits of public support for research. But the method of this survey differs from that of earlier exercises of this kind. What follows is not an account of the well-known centres of excellence in France, but is based on conversations (often informal) with working scientists in France and elsewhere, as well as on documents in the public domain.

The good news is quickly stated. In the past eight years, there has been a quite remarkable deepening and broadening of basic research in France. Everybody knows of research groups in France with long-standing international reputations — the mathematicians of Nancy and Marseille, particle physicists at Orsay, solid-state physicists at Grenoble and Strasbourg, molecular biologists at Paris, the Pasteur institutes and Strasbourg, genome mappers and geophysicists at Paris, chemists almost everywhere. The psychological effect of the grand experiment is that all research groups can now realistically aspire to excellence of that kind. And most seem to have that ambition, as well as the enthusiasm that may overcome the usual bureaucratic obstacles.

Two other features of the transformation stand out. First, French researchers seem finally to have buried chauvinism in the search for international collaboration. A united Europe, in particular, is now, not tomorrow. Second, more by accident than design, the French government has engineered a bridge between basic and industrial research that is well designed to keep the technological revolution bubbling away in France.

That is the good news. Some of the bad news will be found on the pages following. □



Science in France

This survey has been written by John Maddox, the Editor of *Nature*, and Peter Coles, Paris correspondent, both of whom are grateful to researchers and public officials in France for assistance often given at short notice. Cover photos: Feature-Pix/CNES-CSG/SNCF-Lafontant.

Flies in the ointment

FRENCH research has been a great success, but there are structural weaknesses in the system that cry out for attention. French science is in better shape now than ever, so why is there so much talk of emergency, even crisis? One of the strangest features of France's distinctively explicit way of managing research — every major item of public expenditure is explicitly analysed in advance — is the recognition that rationality can often be the harbinger of muddle.

What follows is a stark list of those features of the research enterprise in France that cry out for attention. To the extent that they amount to complaints against the present system, they represent either the opinions of working scientists or are administrative faults acknowledged as such by the managers of the system.

It is important for an understanding of what should now be done that most working scientists are employed directly by the national government. This applies both to those who work for the *grandes organismes* of research (organizations such as CNRS), who acquired the status of civil servants in the early 1980s, and to those who are university teachers. Only during the past decade has the number of technical people working for industrial companies shown signs of substantial growth.

It is also relevant that the national universities are part of a unified national system, lacking the autonomy with which liberal opinion would endow them. Students are admitted on the strength of their performance in the school-leaving examinations, and the power of the universities to impose further selective tests is limited. (But several universities have been seeking to do just that in the admission season now under way, provoking fierce protests.) And the most sought-after degrees are those whose award is validated nationally, on the basis of an approved curriculum and examination.

Against that background, this is where the problems lie:

■ **Universities.** That the universities are in danger of being overwhelmed by their students is generally acknowledged. Wedded to open access by the memory of the troubles of 1968, the government is making a virtue of necessity (see page 133). One danger is that only the most ingenious and tenacious students will acquire a good education in the circumstances that now obtain. Another is that the assumption that research and scholarship are a crucial part of academic work will become even more of a dead letter than it is already.

The government acknowledges that there are problems, and is doing what it can to solve them (see page 134). Principles of good management would dictate

that there should be a greater devolution of autonomy to the universities; whether the device of the four-year contract between the education ministry and individual universities will have that effect will, in the end, depend on whether the government can sweeten its contracts with extra money.

Meanwhile, the plan to provide extra research funds to academics through the agency of a committee located administratively within the ministry of education seems an invitation to nepotism and soft judgement which have given French universities a bad name in the past. If the universities are to win genuine autonomy (a necessary condition if they are to ease the centre's financial burden by attracting funds from regional governments), then *France will need its own version of a national grant-making body distinct both from the ministry of education and from established organizations such as CNRS.* Why not begin now, before the suspicion becomes entrenched that the ministry of national education, reconciled to a measure of university autonomy, is planning to control them in the long term by research grants?

■ **Student support.** The legend of impoverished students eking out life in Paris garrets is too affectionately regarded to be exorcised quickly, but French inhumanity towards graduate students needs to be abated, especially now that the ministry of national education has acknowledged that it will not be able to teach students in future decades without more university teachers qualified at the PhD level.

The standard route to academic life is through the qualification of the one-year DEA (Diplôme d'Etudes Approfondies), involving (for researchers) laboratory work. Student support through this period, after a first degree, is hazardous enough, but university DEA committees, usually organized on a disciplinary basis, become the means by which students are chosen to receive grants to continue with a PhD course.

The numbers of grants seem invariably less than the justifiable demand. Often there will be two or three grants to distribute among 30 reasonably qualified students. Those who administer the system are humiliated that they rarely know in advance how many there will be. They and laboratory directors are embarrassed that so many disappointed students embark on research degrees in great hardship. *If the government wants more "docteurs", why does it not ameliorate the hardships of those who would meet its needs?*

■ **Mobility and employment.** One complaint is that researchers, both established

people and graduate students, are much less mobile than is good for the French research enterprise. Another is that France lacks a system of postdoctoral fellowships for keeping new PhDs at work while they cut their professional teeth and decide, if successful, what fields they will follow. A third is that scientists joining the research organizations are underpaid, and have to wait too long for promotion.

All three problems are linked, and require a common solution. The habit of immobility is engendered by the system requiring that students seeking a research degree must first acquire a DEA qualification, which entails close relationships with research laboratories at which, as likely as not, success will bring them their first research job.

The lack of postdoctoral fellowships *for French researchers within France* means that the transfer of new techniques from one place to another is impeded, while able and established people cannot quickly acquire the help they need to exploit new ideas. (With a few exceptions, even the most influential research groups in France are much smaller than their opposite numbers elsewhere.)

The solution is so obvious as to be breathtaking. *Why not convert established posts in the research organizations such as CNRS into three-year postdoctoral fellowships tenable wherever in France the holder chooses?*

The result would be that able people would get their first taste of independent research at the laboratories that seem to suit them best, that successful research groups would be better able to recruit the help needed to exploit new opportunities and that successful university researchers would be put on a more equal footing with those at the public laboratories.

This is the simplest change of the system there could be. People already on the first rung of the ladder of public service research would not be displaced or otherwise inconvenienced. There would be no significant extra cost — it would be necessary to endow host laboratories with research expenses, but they arise now when new people start work at the laboratories. And it would be possible for promising research groups successful in the competition for people to become competitively strong in a reasonably short time. One of the glaring defects of the present system is that the best groups are consistently understaffed and overworked.

The management of the public laboratories would no doubt be discommoded by a failure to recruit sufficient postdoctoral help to keep their programmes running, but the other side of that coin is that they would be better able to tell who should afterwards be taken on permanently.

■ **Bureaucracy.** The temptation to suppose that a national government

modelled by Napoleon is rife with bureaucracy should be resisted. In reality, the government is both accessible and informal. Ministers consult interested parties, and seem to take good advice wherever they can find it. And while the law is the law, most regulations are decrees, which can be relatively easily amended.

It is nevertheless irksome, and a considerable impediment to the welfare of the research enterprise, that questions of money and of the appointment of people should habitually require reference to the centre. That people should have to travel to Paris to intercede with officials over the rejection of applications for small amounts of money is similarly humiliating and a waste of time whose value is now appreciated in France more acutely than ever. *Now that devolution is under way (see page 131) cannot the Paris lawyers find a legal mechanism for behaving as if the government's researchers were honest people?*

There is a particular problem over the acquisition of special skills, computer programming for example. Few such people will work in government research because they can do much better elsewhere, but laboratories cannot employ them even on short-term contracts for fear of creating illicit government positions.

POLITICS AND SCIENCE

Chevènement's legacy to the 90s

Was Jean-Pierre Chevènement the cause of the transformation of French science in the past decade, or was he himself the product of a process already under way?

The question is still much debated, for which reason it is important that the tense in which it is asked should not be taken as a sign that Chevènement belongs to history or, worse, is dead. As minister of defence in the French government, he spent several days of last month on an aircraft carrier in the Atlantic. One day last week, with three other ministers, he was present at a passing-out parade at the national police college.

Yet there has never been a period in the recent history of the administration of French science quite like the year beginning with François Mitterrand's election as president on 10 May 1981. During the election, the Socialists had made no secret of their ambitions for research, but the reality of Chevènement's plans for increased spending were nevertheless a surprise for many people. The research budget for 1982 turned out to be 25 per cent greater than that for 1981.

This daring leap forward had been planned in advance by a working group under the chairmanship of Professor François Gros of the Pasteur Institute. Mitterrand spelled out his policy for research at a meeting at the Luxembourg

And there is not enough money to purchase all the software needed from outside contractors, so that far too many researchers double up as their own programmers. *Even in France, there should be a solution.*

■ **Money.** France compels admiration for the changes brought about in the past decade and more in the economy at large, not simply in research. It is now a much more prosperous country, even if there are constant envious looks across the border to the East — at West Germany. *Yet the government may have underestimated the true cost of its ambitions for research.*

The calculation is that no great harm may be done if salaries in the public service are so low that people can easily be tempted away to industry, but that will not be true for ever. Despite the success of schemes for adding to research spending from other sources, there is ample evidence that many able people could do much more were it not for the accentuating lack of operating costs. That, on balance, is not simply a waste of people but is potentially destructive of what must now be the most encouraging feature of French research — the generally growing general conviction that every able person can make his or her mark. □

palace on the eve of the election. In the international competition for discovery, will France be at the front or the back? We may be poor in natural resources, but we are rich in grey matter. That was the point at which Mitterrand promised that research and development expenditure would be increased to 2.5 per cent of the gross domestic product by 1985 — a promise subsequently written into law.

Chevènement's energy and enthusiasm seem to have been crucial. Gros has told how, soon after the election, he had visited Chevènement to explain that, whatever else was done, "the first essential is to change the relations between people" in research; his fear was that the imposition of new structures and tasks on a research community already disillusioned by neglect would bring further alienation. Why, he asked, not organize a great national colloquium at which these issues could be argued out?

The notion was not entirely novel; in 1956, the Mendes-France government had organized a colloquium at Caen. But neither Gros nor anybody else appears to have anticipated the energy that Chevènement would put into the project, which fitted with his own political position in a syndicalist faction of the Socialist Party.

Between October 1981 and the end of the year, no fewer than 31 three-day meet-

Colloquia galore

JEAN-Pierre Chevènement's most memorable achievement may have been his decision to hold a series of regional colloquia (*assizes*) at which members of the scientific community could voice their aspirations and discontents. Certainly, he has many imitators.

Beginning this year, the ministry of research and technology plans a series of such colloquia dealing with particular fields of research. The first of them, this January, launched "cogniscience" on the psychology and neurobiology communities. Earlier



Science minister in the early 80s, Jean-Pierre Chevènement.

this month at Strasbourg, there was a similar meeting on planet Earth.

The objectives are several — to improve coordination in continuing fields of study, to plan how to exploit emerging areas (order and chaos, for example) and to explore the relations between academic studies and their application.

But the Ministry of National Education has now joined in, with a colloquium at the Sorbonne last month which gave the minister, M. Lionel Jospin, the opportunity to repeat Allègre's slogan "Diversity means equality" (see page 133). □

ings were organised throughout France, culminating in a four-day meeting in Paris in mid-January 1982. It is estimated that some 25,000 people took part in the regional meetings.

The organization of this gigantic consultation was carried out by a committee under François Gros, with Dr Philippe Lazar (then the president of the scientific council of INSERM, now its director-general) as vice-president and rapporteur. Even now, Lazar vividly recalls how, after nearly a year's intensive work on the organization of the colloquia, he had been looking forward to a vacation. Instead, Chevènement asked him to move to INSERM right away.

Among others, one of the emergent themes was the importance of research as a means of bridging the economic gap between rich and poor countries, now embodied in the great emphasis in French research on the problems of tropical agriculture. But the overarching theme was that science belongs to the people and is a part of the general culture. □

The heat of competition

ONE should not forget that the republican spirit is alive and well in France, more than 200 years after the French Revolution. The grandest people are called "monsieur", as in "Monsieur le Président". And some of the grandest people every day shake hands with those who sit outside their doors, letting through those with appointments and diverting others elsewhere.

Another practical consequence is the institution of the *concours* — the system of competitive examinations by which people compete for places in the public service (which includes student places at the *grandes écoles*, whose *concours* are anonymous — see page 135) throughout France. The objective is to banish nepotism. One Briton working at an INSERM laboratory fulsomely admires the system, which he says should be adopted everywhere.

But there are obvious drawbacks for the management of laboratories. In CNRS, for example, the competition for places (held twice a year) means that particular laboratories cannot be sure that promising PhDs whom they would wish to fill particular slots will succeed in the national competition for places at the first, or second or even umpteenth attempt. Successful directors must have a supply of soft money (perhaps from industrial contracts) up their sleeves.

Promotion is also determined by *concours* — and the chance of success by the low ratio of vacancies to applicants. The obstacles to promotion to the status of *directeur de recherche* (group leader) is one of the chief discontents of young research workers in the public service.

The tendency towards centralism in France, everywhere apparent, also has other origins, mostly Napoleonic. The disadvantages of centralism have traditionally been freely acknowledged, but people have then shrugged their shoulders as if to say that little can be done about them. One of the achievements of the second Mitterrand presidency is that the cosy conventions of centralism are being eroded. The calculation seems to be that French economic growth will be faster if devolution gives regions an influence on their own welfare. That seems to be correct in the case of at least one region in the southwest (see page 131).

But decentralization is being worked out in a centralist fashion; there are the same rules for all the regions, while the devolution of responsibility for education, planning and economic development is far from complete. But in the long run, regional involvement in educational matters could be crucial for research. □

"We want to play the game"

DIRECTOR J. C. Duplessey at the CNRS Radioactive Tracer Laboratory (Institut des Faibles Radioactivités) tells how, when he published his first paper in an English-language US journal in 1971, his professor told him that he had been stupid. "Nobody will read it", he was told. But now the boot is on the other foot. "We want to play the game, and we have learned how to do it."

Where and how to publish is on everybody's mind, but there seems only one answer: in English, and in an international journal. The apparently serious suggestions of a few years ago that people's performance should be determined by a points system, with so many points for a paper in journal X, and so many fewer for a paper in journal Y, have mercifully now been dropped. But there is a general understanding that there is no general case for publishing in French in a French-language journal. Then you will not be read by the people you need to reach.

Nor will you be respected by your colleagues and compatriots. The annual reports of many public laboratories include not merely lists of the papers published during the past year, but an analysis of the proportion appearing in foreign-language journals (see, for example, the figure below).

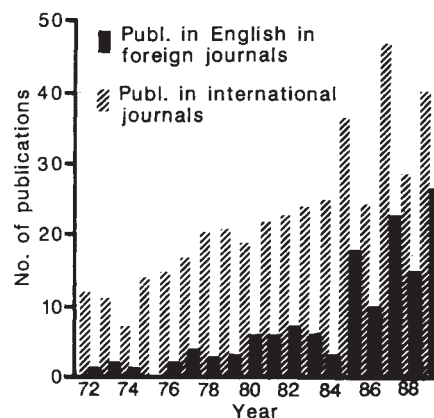
Yet as things are, the change of practice seems to have made little impact on the bibliometric statistics. Recently published figures suggest that between 1981 and 1986 inclusive, the French share of the world's literature fluctuated narrowly and, if anything, that it declined (Martin *et al. Science and Public Policy* 17, 14–26; February 1990). In 1981, the French share of publications in the US National Science Foundation's file of journals was 5.02 per cent, but had declined to 4.84 per cent in 1984, rising to 4.87 per cent in 1988. (The corresponding figures for the United Kingdom are 8.34 per cent, 8.16 per cent and 8.19 per cent.)

No doubt these figures should be regarded with caution. For one thing, they are inevitably out of date. For another, there is plenty of anecdotal evidence — references to outstanding French papers in well-known journals — to suggest that the benefits of the new attitude towards publication have not yet worked their way through. There is also some weight in the observation of one laboratory director that it was fruitless to go looking for evidence of a resurgence of French science "when we may simply have started publishing in English". But the habit has come to stay.

That the French reputation for chauvinism should be so directly belied is proof of a profound sea-change of opinion, linked with a general appreciation that

even the French government's generosity towards research will not enable France to make its own way in a competitive world without external collaboration. So people are also forever on the lookout for opportunities for collaboration, perhaps by the judicious placing of postdoctoral fellows in overseas laboratories, perhaps through collaborations with opposite numbers elsewhere and also through the European Communities.

But compliance does not mean pleasure. For one thing, there are practical difficulties. People whose mother-tongue is French do not write easily in English. (This may be one explanation for the good sprinkling of English-speakers, American or British, in the public labora-



tories.) More contentiously, it is also asserted that French is intrinsically more capable of clarity — which may be another way of saying the same thing.

In any case, most people seem to hold that while English is now the language in which they must write papers — as it has also become a passable lingua franca of conversation — it will be a matter of great regret if French is altogether abandoned. Philip Lazar, the reflective director-general of INSERM, says he hopes that "the time will never come when no science is written in French".

Nor need it. In contrast with those of the English-speaking world, French researchers still regard the production of books as an honourable occupation. Journals such as *La Recherche*, whose strong suit is high-level popularization, appear to be as much a pleasure to write for as they are to read. And the general popularity of technology as well as the need for interprofessional communication has been a powerful impetus for French technical publishing at all levels.

Yet the clinching sign that the use of English has come to stay must be that the *Comptes Rendus de l'Académie des Sciences* now precedes everything it publishes with an extended abstract in English. □

RESEARCH SPENDING

Whatever happened in 1986?

CAN you see politics in a graph? Yes, if it is a graph of French spending on civil research (see right) The 'accordion' effect, as the French say, of policy changes in 1986 and, then, 1988 cries out for explanation.

The present period is one of renewed growth after the cutbacks between 1986 and 1988, but the French research budget is only just finding a character of its own. State research spending, and how this is reflected at the laboratory bench, still bears the imprint of the exuberant policies of the young Socialist government elected in 1981 and then the sober pruning of a neo-Gaullist government that held office for two years from 1986.

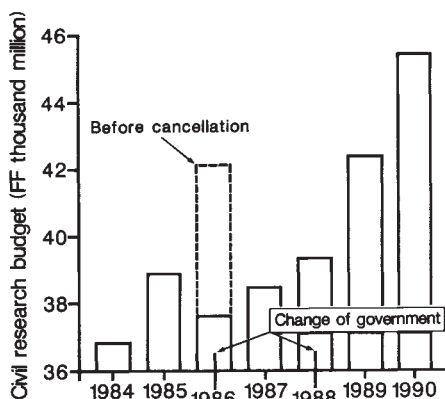
But the tone of the decade as a whole is undeniably that set by the Socialist government formed by the newly elected president, François Mitterrand. In 1981, his new Minister for Research and Industry, Jean-Pierre Chevènement, organized a series of now historic colloquia (*assizes*) to help determine the shape of research policy, spawning radical initiatives that found their way onto the statute book in the 15 July 1982 law on the orientation and programming of science and technology research.

The government declared that civil research spending should rise to 2.5 per cent of GDP (gross domestic product) by 1985. To that end, the 1982 statute committed the government to increase the civil research budget by a remarkable average of 17.8 per cent a year and to increase the number of state-supported researchers by 4.5 per cent each year until 1985.

In 1983, Hubert Curien, the present Minister for Research and Technology (see next page) took over from Chevènement, and innovative schemes were announced to encourage technological progress. A tax incentive scheme to

promote industrial research was set up with the goal that, by 1985, industry should contribute 1.5 per cent of GDP to research and development.

EUREKA, the now highly successful European Communities initiative to promote trans-frontier collaboration in high-



technology industry, was proposed in 1985 by Mitterrand, but it was Curien's brainchild.

It would be facile to say that this early period was uniquely good and that it came to a sudden stop when, in March 1986, the Socialists lost power. (Mitterrand remained as president, during an uneasy spell of what was called cohabitation.) But the neo-Gaullist government led by Jacques Chirac sought above all to curb public spending and to encourage the private sector to carry more of the burden of research support. The 1986 budget was annulled and the budgets of the large research organizations, such as CNRS and INSERM, virtually frozen.

Whatever the economic merits of the Chirac government's drive to streamline what it regarded as a costly and overweight public sector, those were lean

years for state-supported basic research. CNRS, long regarded as a hive of left-wing trade unionists which had become too large and too powerful, was at the centre of a maelstrom.

The ministries for research and for higher education were merged under Alain Devaquet who, with encouragement from conservatives inside and outside government, launched a plan to reform CNRS. But this failed, leaving the research council's affairs in turmoil for over a year.

Devaquet resigned and was replaced by Jacques Valade, but with a much-diminished ministry; most of the budget had been handed to the minister for industry. In the event, Valade achieved little; his appointment was followed by elections which, in 1988, brought the Socialists back to power, with Michel Rocard as Prime Minister and Hubert Curien once again in charge of research.

After a short period when higher education and research were lumped together, Curien was given his own ministry of research and technology from December 1988. The civil research budget, which groups together state finance from all ministries, was reinstated — and the Chirac government was accused of budgetary deception, using defence spending to mask a fall in civil funding.

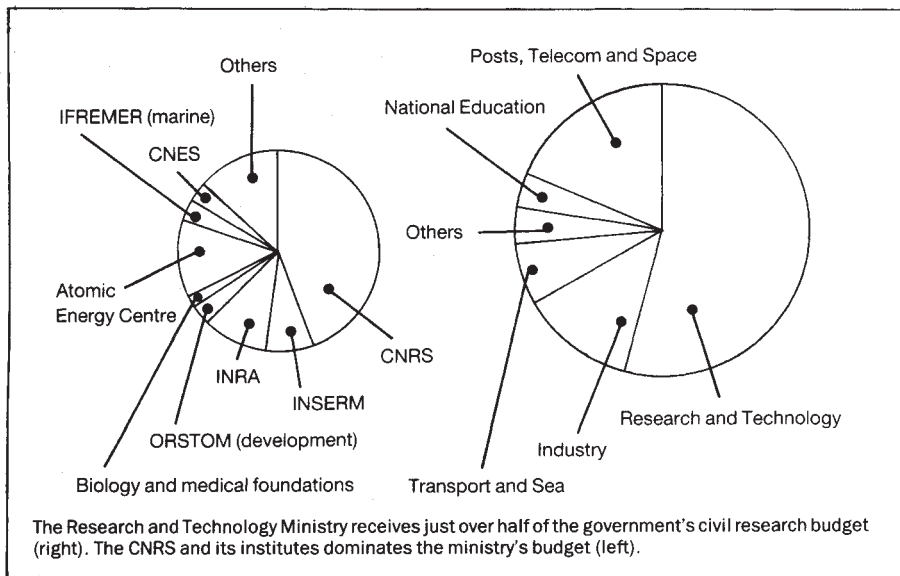
With Mitterrand once again declaring research and education national priorities, Curien was able to inject FF830 million into the research budget immediately to offset the cuts of his predecessor. The 1989 budget was up by 7.6 per cent, recruitment began again and, in the 1990 budget, salaries were improved.

But the growth in the public sector is more like half that of the pre-1985 period. Furthermore, Curien has made it clear that he wants to encourage both basic and applied research, but that big organizations such as the CNRS must run a tighter ship. A committee for evaluation has been set up and plans to 'modernize' the CNRS are under way.

At the research bench, all is not rosy. With inflation running at 3 per cent a year, the 7.3 per cent increase of the 1990 budget amounts to a more modest 4.3 per cent in real terms. The figure is even less when costly space and aeronautics, which were given a special boost, are subtracted. And, because the number of researchers has continued to rise throughout the decade, but faster than budget increases, the average scientist's research money buys almost 20 per cent less than ten years ago.

Yet the present government has not wavered in its objectives. It wants to make sure that France is well placed for the year 2000. And it now aims to see national civil research spending — hovering around 2.3 per cent of GDP — rise to 3 per cent.

Peter Coles



Curien weaves beguiling web

M. HUBERT Curien is an unassuming, almost shy, man with the qualities of a terrier. He was summoned from his post as director of the Centre Nationale d'Etudes Spatiales (CNES) to superintend French civil science on the break-up of Chevènement's ministry of industry and research in 1983. During the Chirac interlude (1986–88), he was elected president of the European Science Foundation, but then summoned back to Paris as minister of research and technology when the Socialist party was returned to power.

Curien believes that French science has, indeed, become stronger over the past decade, partly at least because of his government's willingness to spend money on research. But he insists that research had not been neglected in earlier decades. One subtle change, he believes, is that the French are now proud of their research institutions. In the old days, "we were proud of M. Pasteur, not of the Institut Pasteur."

Links between basic research and industry have also been strengthened, partly at least because of the government's willingness to support (through, for



Unassuming terrier Hubert Curien.

example, CNRS joint projects on a 50:50 basis. Curien is pleased with the way in which CNRS has functioned as a focus for these activities. The interaction has also encouraged French companies to spend more on applied research. "If we had not done this, we should have achieved nothing", Curien says.

Another striking feature of the past decade has been the steady internationalization of science — "it's now a real thing". He believes that French science has benefited powerfully from the impetus that has driven individuals into international collaborations.

He notes that French research groups profit greatly from the relatively small sums available from the European

Commission, whose spending on research has grown to 4 per cent of total European spending on research.

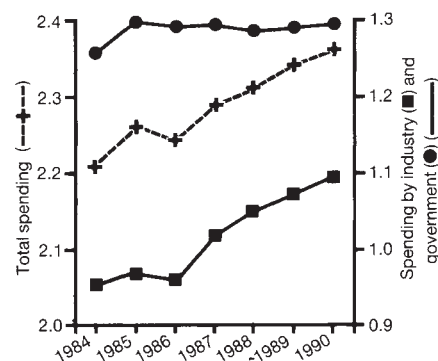
Curien also acknowledges that problems remain. One is mobility or, rather, the lack of it: people tend to stick in the same laboratory or other institution for too long. The concentration on Paris, around which something like a half of all French scientists work, is another.

But except for the salaries of beginning scientists at public laboratories, and the bottleneck in promotion in the middle ranks (which he is trying to widen), Curien is not too worried about the general unflattering comparison between salaries in the public laboratories and elsewhere. "If industry took more good people out of CNRS, I would be happy."

On the general opinion that France needs a substantial scheme of postdoctoral fellowships for its own nationals, he is ambivalent. He acknowledges that postdoctoral fellowships would encourage mobility within the network of government laboratories. Such positions would also help to improve teaching and research at the universities. But, like many others, he is impressed by the experience of 1969, when the then government agreed with the demands of trade unions that postdoctoral fellows of long-standing should be given permanent positions. "That", Curien says, "was a catastrophe."

Curien's position is powerful because his ministry is powerfully placed at the centre of the scheme of things. Other ministries (except defence) must say what they spend each year, and how. This enables him not merely to shape the general balance of research activity but also to back small-scale initiatives. A few months ago, for example, he was putting his weight behind a scheme due to Jean-Pierre Changeux, professor at the Institut Pasteur, for marrying French neurobiology with mathematical studies of the properties of neural networks; according to Changeux, he had mentioned the goal to Curien at a chance meeting. And about now, Curien will be giving a final opinion on an attempt to define French involvement in the Human Genome Project. (Many influential people are now less cool than previously.)

Others of Curien's schemes are less particular. Thus he began a programme (now costing more than FF1,000 million a year) enabling intending researchers, many of them industrial engineers, to write PhD theses by means of a three-cornered partnership with a public or university laboratory and a company. More recently, he has set up a national committee for the systematic evaluation of French science and technology, which



Figures (% GDP) reveal that industry takes the credit for much of the recent increase in spending on research.

in turn has spawned a small unit called l'Observatoire des Sciences et des Techniques, bent on the development of indices of performance.

Yet one of the ministry's goals remains beyond Curien's reach. It has long been the Socialist government's view that research spending should amount to 3 per cent of GDP, putting France on a par with West Germany, the United States and Japan. This year, the percentage is 2.38 per cent (see figure). The ministry blames the shortfall on France's industrial enterprises, whose spending on research has grown, but not as quickly as the government asks. But the figure shows that it is the government's spending that has stagnated, as a percentage of GDP, at just under 1.30 per cent.

But Curien's ministry does more than merely administer science and technology; it is one of the chief means of persuading France's taxpayers (other than those who get tax credits for research spending) that science and technology are good for them.



Pride of France: the Pasteur has at last ousted Pasteur in the scientific hierarchy.

Thus a recent popular (and handsome) booklet on French science under the title "A new impetus (*élan*) for research" echoes the president of France, François Mitterrand, by declaring that its goal is to "make research a national priority". Research, it declares, "is at the heart of the development of modern societies. . .". Research will enable France to "meet the challenges with which the national economy will be confronted in the global market" and to "keep its place among the principal industrial powers of the planet".

Nobody at the research ministry is shy of this rhetoric, which everybody believes.

EMPLOYMENT

Working on the public payroll

ONE way or another, most French researchers work for the government. There are more than 22,000 of them (and 37,000 support staff) on the payrolls of the research councils and the other public organizations active in civil research. (The Commissariat à l'Energie Atomique or CEA, for example, employs more than 1,400 people on research as well as 4,000 on research support.) The numbers of qualified scientists and engineers at the universities may be even greater.

For what it is worth, employment in the public service is growing overall. This year, the research ministry estimates that 432 new posts in research have been created (offset by the loss of 31 posts at the CEA). This amounts to a growth of research posts of 1.9 per cent. The growth of support staff between 1989 and 1990 was roughly half as much.

Luckily, the members of this small army of people do not all behave as civil servants. The republican spirit, as well as the common name *chercheur*, gives them independence. Many are prepared to bite the hand that ultimately feeds them.

Otherwise, industrial research and development is a growing source of employment, as are international organizations as different as the Institut Laue Langevin at Grenoble (see page 137) and the Joint European Torus (the European Commission's thermonuclear fusion laboratory) at Culham in Britain. One also senses among French researchers a growing interest in working at public research laboratories elsewhere in Europe; the Max-Planck institutes are most often mentioned.

In France itself, there is also a handful of institutions which, while largely supported by government funds, enjoy an exceptional degree of autonomy acquired either by tradition or by access to an independent source of funds. One obvious example is the Collège de France, created in 1530 by the then monarch François I to enable him to keep up with the scholarship

And, of course, there are many in research who hold that the grand phrases are merely a statement of the obvious. But the French research ministry differs from comparable organizations elsewhere in taking such a positive stand in favour of the ways in which it spends its budget funds. It is good for the morale of those who work in the ministry's dependent organizations, and may even have helped to cultivate the general French interest in all things new and technological. Curien, who is in no sense a salesman in the conventional mould, may have done more than he imagines to engender enthusiasm for French research. □

often "derisory") by part-time university teaching. The present salary structure in CNRS strongly emphasizes the importance of the promotion barriers in the determination of researchers' well-being.

So the research ministry had promised a three-year promotion plan that will loosen the structure. Beginning in 1991, those who have spent four years on the lowest rung of the research ladder (in the grade *chargé de recherche de deuxième classe*, or CR1), will be promoted to CR1 against evidence of their competence in research. The budget for the current year also allows for a shift in the balance between the three senior and two junior research grades, the effect of which will be to replace 462 CR2 research posts by a similar number in the senior grades (DR1, DR2 and DRE, where DR means "*directeur de recherche*" and E stands for "*exceptionnel*").

There are similar proposals for improving the promotion prospects of support staff, so that an extra FF120million is spent on salaries this year. Yet the employment of support staff is a widespread headache for laboratory directors. Even secretarial jobs must be filled competitively, by *concours*. Technician-engineers are usually able to do better for themselves in commercial industry than in the public service. Computer experts are almost impossible to recruit on the salary scales on offer, with the consequence that many researchers are reconciled to writing their own computer programs (while others skew their industrial collaborations so as to get help of this kind). □

ORGANIZATIONS

Playing the name game

ACRONYMISM has become part of French culture, the influence of the Académie Française on the language notwithstanding. Prudent organizations publish lists showing what the acronymic names for their dependent units mean. What follows is a guide to the principal French research agencies and their dependent parts.

Organizations in France quickly acquire a life of their own. To create a new organization within the public service is no easy matter: the whole government has a say, and the Council of Ministers must approve. But once created, an organization cannot easily be dissolved. Both the bureaucracy and the unions will use their influence in the interests of permanence.

The Centre National de la Recherche Scientifique (CNRS), which celebrated its fiftieth anniversary last year, is the oldest and the largest. Its purpose is the conduct and encouragement of basic research in the whole of science. To that end, it employs 26,000 people, more than 11,000 of them researchers. Its public subvention in the current year is FF10,330 million, up 6.9 per cent from the previous year.

INSERM stands for Institut National de la Santé et de la Recherche Médicale and operates on a scale roughly one-sixth that of CNRS. The current budget (FF1,830 million, up 5.6 per cent from 1989) allows the employment of more than 1,900 researchers. Many of INSERM's research units are free-standing laboratories, more often (as at Lyon, for example) sited near hospital complexes than university student campuses. The interests of these two research councils deliberately overlap. CNRS takes the whole of research for its canvas, but there are several joint CNRS/INSERM research units.

INRA (for Institut National de la Recherche Agronomique) will cost the research ministry FF2,450 million this year (an increase of 5.0 per cent). Its operations are more self-contained than those of CNRS and INSERM, both of which are increasingly dependent for research funds on partnerships with other agencies and industrial organizations. Its 1,670 researchers are only one-sixth of its total manpower, reflecting INRA's role in providing agricultural extension services. □

Money oils the wheels

SINCE 1983, France has offered industrial and commercial enterprises an abatement of their tax bills on account of the research they carry out or otherwise support, but on a formula devised by Jean-Pierre Chevènement that must often seem a little like that of the mediaeval torture apparatus called the rack. The tax credit is calculated not on the basis of what enterprises spend in the relevant year, but on the extent to which their research spending has increased from one year to the next.

How well has the system worked? A study last year by two industrialists (Jean Cantacouzene, director of research at the oil-company Total, and Pascal Gendreau, joint director-general of the government organization for small and middle-sized business) has shown that the system at least provides a means by which the government can get to learn of the changing pattern of research in different kinds of industries.

One surprising finding is that tax credits tend to be a larger proportion of total research spending in traditional industries, such as furniture and textile manufacture and in food processing, than in high-technology industries, no doubt because the companies choosing to register for credits now have only recently embarked on such research.

The system is also cunningly biased against large enterprises. Of 346 companies with turnover exceeding FF500 million applying for tax credits in 1987, the credits eventually awarded amounted to

INDUSTRIAL RESEARCH

only 2.5 per cent of total research spending, compared with 12.8 per cent for the 3,682 companies with a turnover of less than FF500 million. It also seems that the smaller businesses constitute the fastest growing of the tax authorities' applicants, suggesting that the scheme serves its purpose of spreading the research culture.

The figures brought to light by the tax-credit scheme are part of the basis for the government's assertion that French industry is still spending less on research and development than it should be. The report of the Cantacouzene-Gendreau study concludes that industrial research is some 0.5 per cent of GDP less than it should be if France is to keep up with West Germany and Japan in relative terms. That deficit amounts to FF25,000 million a year at present.

Overall, the numbers of companies benefiting from the tax credit system multiplied fivefold between 1983 and 1989, to an estimated 7,000 (the returns are not yet complete). The cost to the Treasury is estimated to have been FF2,600 million last year — but the cost of administering the system is said to have been "derisory".

For the future, it seems to be agreed that the scheme should be given more publicity, and that the government should agree to make it permanent. Already it has been conceded that companies making substantial research expenditures in a single year should be able to spread the funds over several years. □

More relevant to the choice of this priority is France's conception of itself as Europe's research strategist. There have been several occasions in the past 30 years when France has invested its own funds in huge technical projects it could not hope to carry through on its own, in competition with other players on the international markets, but which have been strategically important for Europe.

Nuclear energy is such a case. Although there has been a brief pause in French reactor construction (see page 139), if — some would say when — there should be a revival of demand for nuclear generating capacity in Europe, French nuclear contractors may be better placed than others to win orders from European utilities.

Much the same has happened in aeronautics and space launching, where French investment in research has been used (together with political persuasion) to prompt the formation of international companies that manufacture civil aircraft and offer space launching facilities (Airbus Industrie and Arianespace respectively). In each case, the objective has been to create a European counterpoise to a *de facto* US monopoly.

French support for HDTV is, rather, directed eastwards. From the outset of the EUREKA scheme, several projects have been directed at the processing of digital representations of video images, the basis of schemes for radically improving video definition. Non-French companies such as Siemens (West Germany) and Philips (Netherlands) have been closely involved from the beginning. But nobody should be surprised if official French enthusiasm for HDTV — the EUREKA rules allow governments to contribute towards companies' research expenses — culminates in a proposal for a joint European venture to head off the Sony's of this world.

The processing of silicon wafers is the research ministry's other immediate goal. The code-name is JESSI (for Joint European Submicron Silicon). The immediate goal is to make a 1-Gbyte memory chip, but the hidden agenda might be to make a *supercomputer* on a single chip. Modest Hubert Curien's ministry will acquire clout (perhaps even kudos) if the enterprise succeeds because of its ability (relative to that of comparable European ministries) to urge its client companies towards investment by offering (sometimes threatening) to spend its own money.

For the rest, the ministry plans to concentrate on small and middle-sized companies, traditionally cared for by a state agency called ANVAR (for Agence Nationale de Valorisation de la Recherche), whose budget has been increased by 10 per cent this year. It will be interesting to see, a year from now, how many of those intermediate companies are in the business of high technology. The guess of 100 per cent will not be far from the mark. □

Can government back winners?

FRANCE has taken a long hard look at Japan's success, and wishes it could follow suit. But, with French industry not willing to shoulder the responsibility for spending what it might, the government has become the initiator of last resort.

That seems to be the philosophy on which the French government is now working, and the explanation for the substantial support for industrial research over the past two years. Between them, the research and the industry ministries will this year spend about FF5,000 million on industrial research of a general character, quite apart from the best part of FF6,000 million spent on research at the CEA and other mission-orientated research organizations concerned with space, aeronautics and the like. The largest single increase in the research ministry's budget for the present year — 30 per cent — takes its general fund for industrial research to FF1,565 million.

There have always been hankerings in that direction. The legend of the French

nuclear energy industry began that way, with a deliberate decision to found a new industry with research and development. Did not France also invent EUREKA (in 1983) as a way of persuading other European states to invest funds in high technology?

Research minister Hubert Curien leaves no room for doubt that the sponsorship of industrial research is his priority for 1990, as it has been for the past two years. The emphasis will be on the search for new products and processes, to which the ministry expects to commit FF300 million this year, on perhaps 40 projects. The chosen fields are in new materials and food technology and biotechnology.

Two other goals have high priority, of which the more ambitious is that France should make a mark in the development of high-definition television (HDTV). It is only a small part of the calculation that France itself is likely to be a natural market for devices that bring the clarity of the cinema-screen into every living room.

To modernize and open up. . .

FRANÇOIS Kourilsky, the molecular biologist from Marseilles who is now director-general of CNRS, was appointed to his post by Hubert Curien in 1988 with firm instructions to "modernize and open up" CNRS. How well does he believe the task is going?

He is anxious that the organization should not be mistaken for the whole of French science, noting that it accounts for less than 19 per cent of the total civil budget. But he is pleased about the growing links with industry. At the latest count, industrial contracts with CNRS laboratories number 2,700, nearly two for each of the 1,300 distinct research units. Their total value is FF1,300 million, more than a tenth of the total budget. There is, he believes, a long way to go before the laboratories will be at a loss to know where next to sell their services.

Industrial contracts seem to serve a wider purpose than to keep the wolf from the door of some CNRS laboratories. To this outsider, one of the wider consequences has been to persuade industrial companies that there is, indeed, some benefit to be won from research and development.

Kourilsky says that, until recently, French industry has not significantly increased the numbers of people it employed on research and development. But now, he says, there is a surge in the recruitment of people, especially in fields such as mathematics, computer science, electronics and chemistry, not to mention molecular biology. Evidently he shares Curien's view that those who work for him should be doubly honoured if they leave to work in industry.

But is there a danger that the balance between contract work and basic research will be too much skewed against the latter? Not as things are, he holds. Perhaps the difficulties will arise when, as the volume of contract work continues to grow, but patchily, some laboratories will find themselves short of people for their core programmes while other, lacking contracts, will have people but only modest research funds.

Kourilsky agrees with Curien (see page 126) that the salaries of young researchers should be improved, but considers that the promotion bottlenecks are "still a problem".

On the scheme to provide university teachers with research funds through a committee within the education ministry, he holds that the source of funds should be independent of the university system. "On that, I diverge from Claude Allègre." The problem is that of evaluation. He regrets that the Napoleonic universities "lack independence".

CNRS differs from most comparable

organizations in the large number (1,300) of laboratories in which its people are dispersed. The range is from a handful of people to some scores. So how are new laboratories started, and old laboratories disbanded?

As always, it is easier to start than to stop. The process of evaluation, Kourilsky believes, is accurate and reliable. But the law requires that a decision either to open or close a laboratory should be taken only on the basis of expert advice. The process

BUDGET PRESSURE

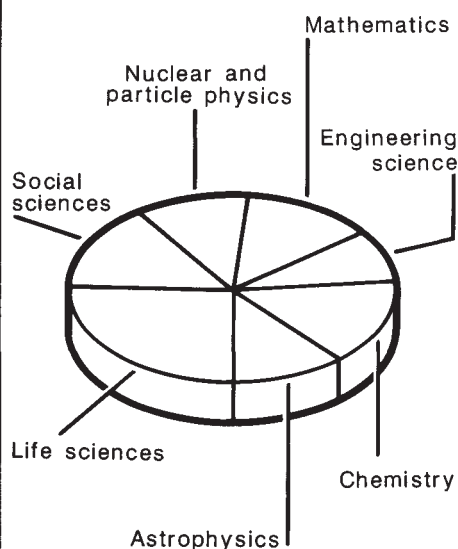
Staying ahead of the game

THE albatross around the neck of CNRS is the risk of being blamed for everything that goes wrong.

Although, on balance, CNRS attracts more praise than protest, the years ahead may be more difficult. The budget squeeze on CNRS's disposable expenditure is one source of strain. The need for some means of financing university research will be a further complication.

CNRS is potentially the more vulnerable because it has a finger in every pie. The existence of INSERM notwithstanding, for example, the life science division (one of seven) takes a quarter of the total budget. But CNRS is also strong in the humanities and social sciences. (The Science de l'Homme et de la Société division takes more than 10 per cent — see figure below.)

Over the decades since the early 1960s, when it was common to find CNRS researchers working in isolation, and on a shoestring, in cubby-hole laboratories throughout the University of Paris, the organization has shown itself to be remarkably resilient and adaptable.



Distribution of CNRS funds between divisions, 1990.

can be slow.

On the future of the organization, he believes that links with universities will be strengthened, together with those with industry. But there are particular opportunities, he believes, in the emerging pattern of a single Europe for forming stronger links with overseas laboratories.

Kourilsky has not been at his job for long enough for his impact on CNRS to have become clear. Moving the bureaucracy will not be a simple task. But he seems to have one important augury on his side — a largely enthusiastic research force. □

The Chevènement upheaval of the early 1980s may nevertheless be one of the best things to have happened to CNRS, confirming its central place in the French scheme of things as well as the role to which it had already aspired of being one of the chief means of research planning in France.

The then-new director-general of CNRS, Pierre Papon, made forward planning his centrepiece. After an elaborate consultation within CNRS and industry, a score (literally 20) research themes were singled out for special attention, and became the basis for research planning in succeeding years. While the interest of the particular themes may since have been attenuated, the mechanism remains. CNRS is forever organizing consultations among interested groups to determine what weight should be given to particular themes.

During the same period, the influence of CNRS on university research has been formalized and legitimized, by the device of associated (*associés*) laboratories, set up by means of formal contracts between universities and CNRS, and in which CNRS and university employees work side by side. (Other research organizations, such as INSERM and CEA, follow the same practice.) Between them, the research agencies have come to provide support — people as well as money — for most of the outstanding research at French universities. Throughout France, CNRS is this year supporting 1,003 research groups by this mechanism. (It also has 366 in-house research units, some of them very small.)

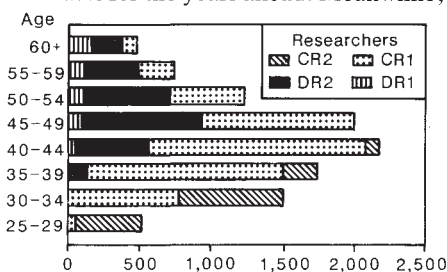
There is no shortage of academic research groups looking for support of this kind. By a curious device invented in 1982, the education ministry nominates (on the advice of CNRS) research groups considered deserving of outside support — *recommandé* is the designation — which may look for such crumbs as fall from the tables of CNRS and the other *grandes organismes* of research, perhaps

in the end for full support. On one reckoning, there are a further 1,000 of these research groups waiting in the wings.

Now, as always, the snag is the budget. Despite the general growth of funds for research, the CNRS share of the civil budget has not grown as quickly as the whole. And the commitment of nearly 75 per cent of CNRS's share to the salaries of its employees means that its disposable income is shrinking. On one estimate, annual research expenses at CNRS account for FF110,000 a researcher, compared with FF180,000 at INRA and FF215,000 at INSERM (the best heeled of the research-support organizations).

CNRS seems bent on righting that imbalance. University research groups, especially the *recommandés*, were chilled at the end of last year when Kourilsky (see previous page) remarked that "CNRS does not have the means to support all university research groups" even when they are excellent. That sharpens the dispute over the education ministry's plan to provide research funds direct, but it also requires that CNRS itself should choose between being the daring cat that steals the cream from the top of the milk and being the pillar of the establishment it has become used to being.

That is for the years ahead. Meanwhile,



Age-structure of CNRS research population, 1988.

CNRS has kept its options open. The organization is typified by its post-Second-World-War headquarters at Gif-sur-Yvette, in the suburbs southwest of Paris. The 'campus' is a park, acquired cheaply in a deal that requires CNRS to keep a team of gardeners on its books so as beautify the park. On one side is a fairly recent chateau (now a visitors' hostel) and, on the other, a collection of laboratories embodying the recent history of CNRS in bricks and concrete (mostly concrete from a less prosperous age). Despite the gardeners, the campus seems seedy.

What CNRS has been doing is plain enough: it has been following trends apparent elsewhere. Although nuclear and particle physics are still responsible for FF790 million, the proportion has fallen from 22 per cent in 1978 to 10.9 per cent in 1989. But life sciences, less than 18 per cent in 1978, are now 25.3 per cent (and employ more than 2,800 researchers at CNRS in-house and out-house units). Inevitably, the life sciences division of CNRS spends more of its budget on recur-

rent costs than on equipment and (less predictably) just about half of its total budget on associated and joint (with other councils) laboratories. In the first half of the 1980s, engineering sciences (especially computers) grew quickly — and the number of research posts virtually doubled — but now chemistry is the most quickly growing.

CNRS has also been ageing. In 1988,

RESEARCH CULTURE

A club-like atmosphere

THOSE who work for CNRS behave like members of a club because they are members of a club. To be a CNRS researcher is to be a member of a distinctive group, as if it were to be the French Navy or the *corps diplomatique*. But CNRS is, of course, not quite as grand.

The sense of club is easily explained: for at least the past decade, entrants to CNRS have won their places in the public service by competition in the common *concours*. They vividly appreciate the distinction between themselves (who are in) and those who are out. At least until recently, they could be reasonably sure that they would work permanently in this branch of the public service, with occasional postings elsewhere.

But this commonality does not engender a dull sense of uniformity. Different research laboratories have their own distinctive spirit. Some are plainly riding high, perhaps sustained by industrial contracts or grants for special projects. Others harbour a deep sense of neglect. They are inevitably the laboratories most oppressed by the bureaucracy of their parent, which is organized into seven divisions and no fewer than 36 sections, each with its own secretariat.

The underlying difficulty is the small proportion of the CNRS budget free for research expenses. More than 75 per cent of the budget is committed in advance to salaries; heat, light and maintenance take a substantial proportion of the rest. (INSERM seems better placed in this regard.)

Travel funds are not too hard to come by; most people acknowledge they can make one substantial overseas journey every year or two, while European Communities sources are increasingly used for travel within Europe. But the proportion of a laboratory's total budget remaining in a director's hands for research expenses may be relatively very small, perhaps less than 10 per cent of his salary bill.

Yet CNRS abounds with people who hold the highest opinions of their organization. For many, the sense of security and freedom they enjoy makes up for the gap between government and industrial salaries. One, the director of a laboratory

only 35 per cent of its researchers were younger than 40. Although the recruitment and promotion rates are now to be increased, it is difficult to see them quickly creating the bottom-heavy pyramidal age-structure one would expect to find in a research organization. No wonder that Curien (see page 126) says that he is glad that industry threatens to hire away senior people. □

at Gif-sur-Yvette, says that CNRS provides unique opportunities for research. Another gives it as his opinion that CNRS, whatever its faults, has become the dominant arbiter of quality in French science. The organization's willingness to enter into collaborations with universities and industrial companies, sometimes taking the initiative in forming them, is generally commended.

One British member of a joint CNRS/INSERM laboratory says that he is more settled, and more productive, in France than he had been at a British university. He reckons that the continuing shortage of academic positions in Britain must soon stimulate a southwards stampede across the English Channel when British people realize that, at the outset, all the French they need is that required to keep body and soul together.

Another Briton at another laboratory, fresh from a postdoctoral position in the United States and with an enviable reputation for the development of a new technique, says that he chose the CNRS laboratory from among half a dozen at which he had been offered a position. (Sybaritic considerations seem to have played a part in the decision.) Testimonials of this kind abound. Because, to paraphrase *Anna Karenina*, contented employees are all alike, but discontented employees differ in their discontent. The following complaints against the organization are necessarily more voluminous. Their balance should not be judged by the space they occupy.

First, there are problems about people. Laboratories not well-endowed with outside funds are less able than others to keep competent young people at work while they compete for permanent places at successive *concours* for permanent positions. The result, some say, is that able people are often lost to the system at the beginning of their careers.

Filling vacancies is a constant headache. If a vacancy should arise, even among the technical or administrative staff, it cannot automatically be filled. Instead, the section or even division to which the laboratory belongs will at least consider whether the vacancy should instead be awarded to some other unit. According to

at least one director, there are no mechanisms for appointing even junior staff on a temporary basis; either a public servant, or nothing.

There are recurring problems about research allocations. Laboratory chiefs are usually told, in the February of the year to which the information applies, what their allocation of funds will be. One CNRS director says that last year, after his budget had been cut by 30 per cent with no explanation, it took him until November to get the cut restored. (Luckily, the rules do not require that funds should be fully spent in the year for which they are intended.)

The need to make visits to Paris regularly, either as a member of an advisory committee (which is essential for staying in the swim) or to lobby against some particular injustice, is particularly irksome for those who live and work elsewhere. Reimbursement for the cost of doing so, as for other expenses legiti-

PLANT SCIENCE

mately incurred on CNRS business, is generally agreed to take months. One researcher says that he expects that CNRS will at any stage owe him FF20,000, or a month's salary.

Although many CNRS laboratories on university campuses enjoy the respect of and a good deal of influence with the associated universities, others lead a precarious existence there. One such group has been required to move its laboratory several times in the past seven years to suit the convenience of the university, and is now threatened with another move in October.

The most serious of the complaints against CNRS is that at least some laboratories appear to be inadequately informed of the overall policies that determine their affairs. This seems a shame when the people concerned are every bit as full of enthusiasm for their work as the general run of people working in CNRS laboratories. □

Hungarian sowing seeds

THE Institut des Sciences Végétales at Gif-sur-Yvette differs from others in the park in that it is brand new and that its director, Adam Kondorosi, is newly arrived, with his wife and co-worker, from Hungary.

The refurbished laboratory is that previously occupied by the teams who danced attendance on Gif's giant phytotrons, which were in the 1960s proof of CNRS's promise to botanists that they are deserving of large equipment as are nuclear physicists. The phytotrons have now been dismantled and the laboratory disbanded.

Kondorosi has been commuting between Gif and Szeged in Hungary for the past 18 months, while recruiting people for the laboratory. His appearance, more or less permanently, in this Paris suburb justifies earlier fears in Hungary that liberalization would entail the loss of able people (see *Nature* 344, 611; 12 April 1990).

Kondorosi says that he is enjoying himself enormously, but that whether he will stay for good depends as much on his wife's inclinations as his own. Meanwhile, he says that he has continuing responsibilities at Szeged, mostly for the continuation of his research with graduate students, so that he will have to make occasional visits. CNRS seems compliant.

For the rest, Kondorosi's tale is an illustration of how CNRS (in this case with some help from INRA) sets about founding a new institute. The impetus was the conviction that the molecular and cell biology of plants would have important applications in agriculture. An international committee spent some months head-hunting for a director until it found

Kondorosi (he rattles off the names of those who turned down the job). Now the objective is to recruit a team of people, perhaps internationally, to staff the five research groups whose work will stamp the laboratory's programme (and whose heads are already in place). The hope is to build up to between 60 and 80 people in four years.

One distinctive feature of the programme is that external collaboration is to be built in from the outset. Kondorosi says there are already agreements with the Max-Planck Institute at Köln and the department of molecular biology at Rome, not to mention his old institute in Hungary. He is also planning to work closely with the complementary CNRS institute at Toulouse, and is hoping for further support from the European Communities and from the European Molecular Biology Organization.

Kondorosi's own field is the study of how the symbiosis of rhizobia and plant roots is reflected in and mediated by the reciprocal exchange of chemical signals between them, which is one leg of the institute's programme. Another group will aim at similar understanding by more classical studies of the hairy-root syndrome (a variant of crown gall disease). There are also groups concerned with plant cell signalling (by means of auxin), the mechanism of damage by plant viruses and the molecular genetics of *Arabidopsis* — the eukaryotic plant with the smallest genome so far recognized.

Like other up-and-coming CNRS laboratories, the new plant sciences institute is already embarking on graduate education. Five or six students seeking the

Regional consuls in the wings

MME Katherine Piquet-Gauthier is the new regional delegate of CNRS at Montpellier, with responsibility for CNRS affairs in Languedoc-Roussillon in the south-west of France. She is one of a dozen people appointed last May, under the terms of a decree promulgated by CNRS last December, to offer regional groupings of laboratories with a more immediate presence than Paris can provide. But it is not yet clear how far administrative devolution will go.

The twelve regions and the sites of CNRS's regional offices are Alsace (Strasbourg), Aquitaine and Poitou-Charentes (Bordeaux), Brittany and Loire (Rennes), Limousin (Orleans), Ile de France (see below), Languedoc-Roussillon (Montpellier), Lorraine-Ardenne (Nancy), Midi-Pyrénées (Toulouse), Normandy (Caen), Provence-Côte d'Azur (Marseille) and Rhône-Alpes (Lyon). The administrative centre for the Pas de Calais region had not last month been chosen.

Many of the regional directors have not yet taken up their posts. The arrangements for the Ile de France (greater Paris) are complicated by the great concentration of CNRS responsibilities in the region, with the result that there will be five sub-regional directors working through a central office. Similarly, there will be two sub-offices in the Rhône-Alpes region catering separately for Lyon and Grenoble.

What will the regional offices and their directors be able to accomplish? Laboratory directors and researchers have a variety of aspirations for the new arrangements. Those who labour under a sense of neglect hope the new arrangements will give them a stronger voice in Paris. More immediately, there is also the prospect that the regional offices will simplify the bureaucratic structure (although some fear that their influence may be in the other direction).

On the question of whether devolution will make it easier to pay people's legitimate travelling expenses more quickly, Mme Piquet-Gauthier says "not yet, but wait and see what it's like when you're here next". □

DEA diploma (a pre-PhD qualification — see page 122) have already been accepted for next year. Kondorosi expects that university relations will be simplified because two of his group leaders are also university professors.

But will there be enough teachers for them? Kondorosi says he has already advertised 18 vacant positions at the institute. There can hardly be a quicker way for someone from Eastern Europe to learn the ways of the competitive West than he has chosen. □

CNRS reflects with pleasure

CNRS, which is always evaluating other people, has recently been evaluating itself. But the result is not so much a critical appraisal of the largest single research programme in France, as a kind of guidebook to science in general. The report of the evaluation is dominated by the theme of the growth of interdisciplinary research requiring multidisciplinary teams.

The self-evaluation, the report of which has just been published as *Rapport de Conjoncture*, has been prepared by 22 commissions established to evaluate themes singled out by CNRS for study, and ranging from Earth and Solar System to order and chaos and the transformation of societies. Readers must be prepared to look between the lines for outright self-criticism.

But one general theme is the emergence of a number of interdisciplinary problems to which French science should pay attention. One singled out for discussion is that of global change, but the study also notes that the properties of neurons promise to be of great importance in computer sciences as well as in physics and mathematics.

This colours the report's opinion of present arrangements for the training and recruitment of young people into science. It argues that there is a need to improve and increase the training of doctoral students to ensure a sufficient supply of researchers and university teachers. But doctoral training should also prepare young researchers for working alongside people of other disciplines. The report asks that something should be done to improve the public image of certain disciplines, citing mathematics (surprisingly) and chemistry as being unpopular.

On interdisciplinary research, most conspicuously at present represented in

CNRS by four named research programmes (in materials science, energy, environment and technology at the workplace), the evaluation draws attention to some of the problems faced by those engaged on them, in particular by the risk that work on interdisciplinary problems may complicate the two-year evaluation of their research achievements by one or other of the 49 subcommissions, are responsible for this work. The report argues that people's willingness to work on interdisciplinary projects should be regarded, *a priori*, as favourable for their careers.

Of the international connections of French research, the evaluation acknowledges that English has now become the language of communication in international science, but says that there remains room for French on the national scene and in relations with francophone countries.

In general, the evaluation says, it has taken an optimistic view of the place of French science on the international stage, but it acknowledges that there may be a bias in the process of evaluation — research that is inherently good will be well spoken of, while that which is bad will leave little impression on outside critics.

The document acknowledges frankly that international collaboration entails the risk that researchers will be lost to France by means of a "brain drain" which is not confined to France. But it says that French collaboration in large international projects has an effect "at once dynamic and stabilizing". It says that the involvement of French researchers in overseas laboratories, and visits of overseas researchers to France, but particularly the exchange of scientists within Europe, "must be one of the keys to our successful development".

Some rights and wrongs

AMONG the points made in CNRS's evaluation of itself are the following:

French groups have played an important part in the development of **particle detectors** at CERN and DESY since the success with Gargamelle in 1973, but steps should be taken to build on the experience of constructing the LEP injector at Orsay. Building a **4 GeV** electron accelerator, "no doubt in a European context", would allow important work with high-energy photons in **nuclear physics**. New instruments, in Hawaii and Chile will be followed by the Very Large Telescope (four linked 8-metre telescopes) being built by the European Southern Observatory, but French **astronomy** should be primarily concerned with the supply of theoreticians.

More generally, the subcommission on these topics expressed concern at the difficulty of preparing graduate students both theoretically and with a capacity to tackle unknown problems, with the "linear" careers of young researchers and with the continuing shortage of research funds.

The mathematics evaluation says the French **mathematics** school is "probably one of the best in the world" (which, by general consent, is fair). There are up to 2,500 mathematicians at universities, with only a tenth as many at CNRS. Now, the evaluation says, CNRS should be employing more mathematicians who are, it is said, less and less easily classified as "pure" and "applied". By way of proof, the evaluation draws attention to the invention, by engineer Jean Morlet, of the concept of wavelets (*ondelettes*, or convolutions of periodic and gaussian function as sets of elementary functions in which seismic signals can be expressed.)

Surface research is crucial in, for example, the electronics industry, and "France is now well-placed" by the provision of national facilities by collaboration between government agencies and industry. But the advantage could be in hazard because of the shortage of CNRS funds for medium sized equipment, while the immobility of people makes the development of effective research groups difficult.

In *informatique*, France is said to be strongest on the theoretical side and in the development of languages, while Japanese and US industrial laboratories dominate such fields as the development of an **optical computer**. Yet in both respects, the evaluation says that European collaborative programmes have a crucial role. CNRS is said not to have increased its numbers of specialists in these fields sufficiently. □

Living better by one's wits

THE Napoleonic centre, it appears, can be flexible enough when there is cash to be saved. That seems to be why every laboratory in the public service seems to have a freebooter's licence to strike a deal on research with some third party, within the limits of a framework agreement. So laboratory directors at CNRS and others of *grandes organismes* have become entrepreneurs of a kind.

The results have often been spectacular. In some CNRS divisions, external support may be as much as a third of the total. Other ministries and public agencies provide more than half of this extra money, for projects as different as the provision of special research equipment and for research projects with a bearing on some development by a still nationalized

industry — the High-Speed Train, for example. But particle and nuclear physics and the life sciences are almost exclusively financed out of CNRS funds.

It is also striking that France derives a substantial research support from international sources — in 1987, this amounted to 17.9 per cent of CNRS's disposable income, exactly the value of research contracts with industry. The European Community is a particularly important source of funds, both because participation in the major European research programmes carries public researchers into joint projects with industrial researchers and because the much smaller sums available for occasional travel from Community sources valuably supplement what CNRS can spend. □

A search for *égalité* in diversity

CLAUDE Allègre, the bluntly spoken geophysicist who was director of the Institut du Physique du Globe at Paris VI until two years ago, could make his name with the quiet revolution in higher education on which he is now engaged. Or he could lose his academic friends.

Allègre, energetic and at once ambitious and impatient, has become director of higher education at the Ministère de l'Éducation Nationale (and de la Jeunesse at des Sports). But he keeps his hand in at the institute. He teaches a course and spends time in the laboratory. He hopes to manage every weekday morning next year. But even now he is working like a dog.

The task is herculean. By any standards, the university programme is calculated to astonish. In the academic year just ending, there have been 1.13 million students (or their equivalents as part-timers) at France's 76 universities, which means that 40 per cent of school-leavers at least begin on higher education. Allègre says that the ratio should be 60 per cent. "Then we shall be like the United States and Japan."

There is another driving force. The



Down to Earth: Allègre is keeping one foot in the university world.

university system is what it is because of the student rebellion in the streets of Paris in the spring of 1968. Nobody forgets that the Left Bank was occupied by students for days on end. (Many of those same students are now, no doubt, civil servants.) Thus has been accepted the principle of open access: qualify in *le bac*, the popular name for the *baccalauréat*, the nationally standardized school-leaving examination, and there will be a place for you at a university. Naturally, the numbers grow.

So, inevitably, does the budget. In real terms (francs of constant value), spending

on higher education has increased by 35 per cent since 1980, but half that increase has been squeezed into the past two years. The French government will have spent FF27,500 million (in present money) on higher education in the current year. Next year (the budget is just now being devised) it will no doubt spend more.

Allègre is not one to be dismayed by the immensity of his responsibility. He spells out the agenda. Build more universities (eight are planned, four in the greater Paris region called the Ile de France, four at places yet to be determined). Lift the spirits and the public reputation of academics by increasing the pay of able people, perhaps by 25 per cent. Recruit more graduate students, perhaps by offering them better stipends. Improve the quality of academic research by a system of competitive research grants administered by the ministry. Sign contracts with universities so that they can spend their budgets to the best advantage.

There are even plans to change *le bac*. Allègre is proud of a scheme to introduce students to chemistry by asking them to build molecular models; by the time they come to grapple with the laws of multiple proportions and the like, they will at least know what is meant by valency. There are also plans to introduce continuous assessment of students' performance in the awarding of *le bac*, if not in mathematics, then in other fields.

All this is meant to happen quietly. "We are making a revolution", but we say that "nothing much is happening". The more natural course would have been a "big new law", but that tendency is the "French disease", Allègre says.

Allègre does not hide from difficulties, but meets them head-on. Will not a system in which universities' activities are predetermined by a contract with the ministry mean that university freedom is curtailed even more than at present? On the contrary, says Allègre. Universities knowing what they want to do can negotiate an appropriate contract with the ministry, and then be more free than ever to pursue their goals.

He is passionate on this point, complaining that his republican compatriots have consistently failed to appreciate that diversity means *égalité*, and is not antithetical to it. The tendency has been to suppose that *égalité* requires that everybody should follow the same curriculum at the same kind of school. Emphasizing that the participation rate should be high (60 per cent) he says, "Look here, France is a Catholic country, and Jacobin and Napoleonic. . . in the end, the Catholic influence has won through."

Those are the forces in favour of uniformity and centralism. He acknowledges

A new vision or an old mirage?

ACADEMICS in France differ in their estimates of whether the government's new promises of support will materialize, but only because there are different degrees of scepticism. Most say, "We have heard all this before. We'll wait and see what happens." Many others will not wait.

What follows is honest reporting of academics' views adapted so as to conceal its sources, for reasons that should be obvious. (It matters less that academics are literally civil servants than that their senior colleagues can still powerfully influence their academic prospects.)

One group of 40 university teachers in literature — a group now responsible for the first-year education of 700 students — is asked (by the university administration) to take responsibility for 1,200 students. That would mean increasing the student to staff ratio from 20:1 to 30:1. There would be no extra teachers.

A teaching group of similar size, most of whose members applied for special contracts on the grounds of special expertise in teaching or research, has found that only two special contracts have so far been awarded. The recipients are both in their fifties, and are people of acknowledged distinction. One is so eccentric that he no longer teaches, and does not publish either.

There is a general complaint at the teaching loads of university professors; ten hours a week of lectures is commonplace. "That's impossible", says one professor at Paris VI. Such a load means that even the teaching must be skimmed, while there is literally no time for research — and the funds for supporting research are steadily declining.

The position may be "even worse in Britain, but not in West Germany — there they seem to be spending money more intelligently". In France, there is a crisis. There are "not enough teachers, and those there are thinking of leaving", mostly for international organizations and industrial laboratories.

Other academics protest that the criteria for deciding promotion within the system are far from objective. A proposal that people's competence as researchers should be judged objectively, perhaps by publications records, was rejected by the ministry a year ago. □

that universities as they are are too much alike. But freely negotiated contracts with the ministry could give universities the right and the opportunity to be different. The first negotiations with universities, in the west and south of France, are now under way within the framework of an elaborate set of parameters conjured by the ministry — 12 m² of space for a science

student, for example, but 3.5 m² for one in law. Individual universities are making special claims in respect of extra things they do.

The contracts, when negotiated, will run for four years which, a little to Allègre's delight, breaks the rule that ministries must not anticipate spending not yet voted by the National Assembly. (One guesses that the ministry's lawyers will deal easily with that subtlety.)

Allègre also agrees that French universities have been too conservative. Scornfully, he notes that molecular biology was taught at and by the Institut Pasteur long before it found its way into the curricula of the universities in Paris and that plate tectonics (his own field) remained in limbo for too long.

The ministry's mechanism for supporting university research is more controversial. Academics from all over France will compete for a share of a pot of money administered by a ministry committee, half of whose members are drawn from European countries other than France. Allègre is fighting for a 15 per cent increase of the funds available in the budget for 1991, now being drafted.

Might not a ministry committee assessing the quality of research proposals be tempted to use the funds at its disposal for solving other than research problems? Would not an independent grant-making

agency be safer? Allègre does not agree. For one thing, the committee is international, but its subcommittees will also rely on CNRS assessments when making their decisions.

As proof that the system is working, Allègre notes that it has already drawn blood. "A lot of people are very upset — mediocre people and some universities." But he does not like the US system of competition for research grants; "indirect costs" are "ridiculous", as is the difficulty of finding support for long-term projects.

He is confident that "zero — nought" will be spent on "big programmes". But he would like to encourage research in innovative fields. He rattles off a string of topics — *informatique* in clinical medicine, molecular identification by physical means, cogniscience (the new name for neurobiology), the geophysics of the topmost 100 metres of the Earth's crust and the meteorology of cities.

So why, with these grand plans, does Allègre risk losing friends? Few will dispute the good sense of his ambitions. One worry is that the interventionist habits of the ministry will persist. More important (see below), there is a general fear that the funds for supporting Allègre's quiet revolution will be, as so often in the past, inadequate for the need and, perhaps, even hijacked by the pressing needs for general support in the university system. □

UNIVERSITY REFORM

On the way to open access

In the academic year just ending, there were 1.13 million students at French universities, an increase of 77,000 over last year. Next year, there will be more — just how many more will be known only when high-school students have been told the results of *le bac* this year.

For several years, the system has been growing at about 8 per cent a year. The mounting pressure of numbers is felt even in the highest reaches of the government — the official communiqué from the Council of Ministers (the French government's cabinet) announced that the building of 200,000 m² of extra space at universities had been sanctioned against the new academic year.

Since 1960, when there were a mere 215,000 students, the scale of French higher education has multiplied more than five times. The growth has been most rapid in the humanities, whose students account for more than a third of the total. Law and the *sciences économiques* (which include accountancy and management) account for more than 250,000 students. But in 1989 there were 192,000 students following courses in science and engineering, an increase of nearly a half since 1980.

As Mr Kingsley Amis would have predicted had he been French, more has

meant worse. Faculties have not grown as quickly as student populations, so that ratios of teachers to students have generally declined. Harassed teachers at institutions other than the *grandes écoles* have been forced to skimp on research and scholarship as teaching and administrative burdens have increased. Who can wonder that many researchers in dedicated research laboratories scorn their nearest-neighbour academic colleagues? Or that, mostly for lack of suitable candidates, there were 2,000 vacant teaching posts in June 1988?

The pressure of numbers is greatest in the early years. Undergraduates follow a three-stage curriculum (the *premier*, *deuxième* and *troisième cycles*). The attrition rate at the end of the first cycle (for many, the end of the first year of studies) is high. For those who stay the course, the *troisième cycle* or fifth year is either a vocational course (such as the preparation for a career in teaching) or, in science fields, a preparation for research.

The education ministry is not for nothing the Ministry of National Education. It operates schemes for the national validation of degrees, the *licence* and *maître* qualifications of the first and second cycles in particular. Those with

academic bent follow a course leading to a DEA (see page 122) qualification, previously a sufficient qualification for higher education work, possibly following that by embarking on a PhD thesis, lasting for two or three years.

The government's plan to put right this state of affairs rests on these components: **Salaries and careers.** Teaching posts will ordinarily be filled only by people with PhD degrees. Teaching salaries will be tied to those in the public service and a system has been instituted by means of which the salaries of academics with special responsibility and competence in research, teaching or administration may be augmented if those concerned are offered special contracts of employment by the education ministry. The ministry says it has received 15,000 applications for such contracts, the effect of which will be to increase the salary of a beginning academic from FF8,000 a month to FF13,000. Allègre (see page 133) reckons that the average uplift of salaries will be 25 per cent.

As part of the process of deliberately preparing people for careers as academics, the ministry has set up thirteen *centres d'initiation à l'enseignement supérieur* at which, it is expected, graduate students seeking careers at universities will attend for one week twice a year during the average three-year duration of a PhD course. Graduate students selected for these programmes will be paid extra (a total of FF9,200 a month), but will be expected to teach classes (usually in the *premier cycle*) and to hold small-group seminars while preparing their theses. The plan is to increase the 3,000 people at present following this programme to a total of 6,000 (implying an annual production of 2,000 academics in all fields); graduates of the *grandes écoles* will be extra.

New universities. Existing universities earn intangible credit at the Ministère National de l'Éducation by their willingness to fall in with the notion of expansion, but there are limits to what can be accomplished on existing sites. Montpellier (see page 138), with 50,000 students, is pondering whether it could take an extra 20,000; one solution would be to spin off first-year teaching to Nîmes, 60 km away. Nîmes, with ambitions to be a university-city in its own right, is not so sure.

So France, almost alone among industrialized countries, is embarked on creating eight new universities — four in Paris and four elsewhere. The new Parisian universities, which will be multidisciplinary, will be suburban universities — at Cergy-Pointoise, Versailles and Mame la Vallée et Evry with a dependent campus at Melun-Senart. But it is also planned to increase by rebuilding the capacity of the Sorbonne and other universities in central Paris.

The hope is that the four new provincial

GRANDES ECOLES

universities, the locations of which have not yet been chosen, are meant partly to meet the demand for higher education which is, even at present, unsatisfied in west and central France. (There is at present a net movement of students towards Paris, the southwest and the Lyon region.) The problem will be to persuade able academics to move away from the centre.

Research. The government acknowledges the need to "arrest the slow erosion of university research over the past several years". The ministry of education hopes to accomplish this by several means.

There will be a system of competitive grants for research and scholarship administered by a committee (the Conseil Scientifique de l'Education Nationale) under the Nobel prizewinning chemist Jean-Marie Lehn. Salary supplements for academic-researchers should work towards the same end.

It may also help that the government plans to encourage the emergence of new university centres of excellence. It has nominated a handful of places which, "by the quality of their research, the diversity of their teaching and the attractiveness of their locations", may rival "Oxford, Heidelberg and Berkeley" in the united Europe of 1993. The ministry has so far nominated Grenoble, Strasbourg, Orsay-Polytechnique (southwest of Paris) and Toulouse; it promises further names — but a few of them — before 1993.

Organization. The ministry of education, at the Napoleonic hub of France, is used to redefining and rebalancing the interests of the centre and periphery. The new calculation is that four-year renegotiable contracts with the ministry of education will give universities an incentive to skimp on spending in fields in which costs are elastic, and to invest the funds they save where there are intellectual opportunities, or students to be recruited. The council of ministers, on the recommendation of the ministry, will continue to appoint the rectors of the universities.

Several innovations are promised. A study is under way (with the ministry of economics and finance) to see whether budgetary procedures can be radically simplified and whether real-time ("*en temps réel*") techniques of data processing can assist the administration of universities. (Ways of counting students would be a big help.)

Cosmetics. The ministry, which hopes that it will be possible to double the number of doctoral students preparing theses in the five years ahead, plans to set up an organization for monitoring all theses under preparation. It is hoped that this information (to be published annually) will form the basis for determining university research policy as well as for comparisons between French and other research. □

Lyon's hot-house university

WHY should French teenagers compete so fiercely to go to universities that are unable, by their constitution, to award degrees? Because the universities are not universities at all, but *grandes écoles*.

These citadels of French higher education owe their existence to functional considerations: how best can the state secure the services of able people who have been specially trained in certain fields? By recruiting them young and competitively, by making them civil servants for at least ten years and then, by training them. Thus was the *École Polytechnique* Napoleon's device for assuring a supply of artillery officers.

By definition, the *grandes écoles* are in Paris, or at least they were. But in the 1980s the ministry of education embarked on an experiment to see whether they could be transplanted to provincial soil. Now, after more than a decade of heart-searching and hard work, a version of the *Ecole Normale Supérieure* has been transplanted from Saint Cloud, in Paris, to a complex of post-Modern buildings on the site of an old abattoir in this provincial city, now the next largest after Marseille.

The *Ecole Normale Supérieure* de Lyon has taken in 100 students in each of the past two years. They are a cut from those successful in the national *concours*. Most give themselves an extra two years preparation after *le bac* before entering the competition, which means that *normaliens* are usually 20 years old when they begin their studies. Although most of those entering the annual competition give one of the Parisian schools as their first choice, they (and their teachers) know they come from the top 0.1 per cent of whatever proxy for the national IQ distribution is measured by the *concours*.

Polytechnic Institutes

THREE relatively new institutions, in many ways intermediate in character between the *grandes écoles* and the national universities, have a particular influence. The best known is the Institut National Polytechnique de Grenoble, with roughly 3,000 students and an associated group of *grandes écoles* specializing in technical fields. There is also an Institut National Polytechnique at Toulouse and another in Lorraine.

All three were founded in the early 1970s as a means of stiffening higher technical education and lending coherence to its academic procedures. All are relatively small in terms of student numbers — between them the institutes have about 8,000 students. □

Moving the school from Saint Cloud to Lyon has not been trouble-free. The idea seems first to have been mooted in the mid-1970s, and more or less agreed by the arrival of the Socialist government in 1981. At that stage, those threatened with banishment from Paris, and their unions, pleaded with the new government to put a stop to the plan. But the then minister of education, having brooded for some time, decided not merely that the move should go ahead, but that Lyon's floor-area should be increased by 11,000 m² to a total of 36,000 m².

Guy Aubert, appointed director of the new school two years ago, is an energetic man; among other things, he reckons to travel 50,000 km a year on the autoroute between his laboratory at Grenoble and the school at Lyon. He has high ambitions, not least that of making Lyon the best of this esoteric bunch, as follows:

■ **To get the best students.** Even at the top, competition remains fierce. Lyon has to stake its claim on the affections of the brightest few hundred making their way to the *grandes écoles* by the reputation it establishes in the next few years. The counter-attractions of Paris will persist. But even as things are, 3,000 people compete for the 100 places at Lyon. Competence in some foreign language is required. French entrants are paid as junior civil servants.

■ **Education through research.** Lyon offers three options — mathematics and *informatique*, science of matter (physics or chemistry) and life and Earth science. From the outset, students at Lyon will be plunged into research (and, during the first two years, taught to write and speak English). The requirements vary from one department to another, but most students spend half of the first three years of the four-year course on laboratory work and research. In the process of doing so, they normally acquire (from the University of Lyon) a first degree and, usually, the *diplôme d'études approfondies* (a necessary but not sufficient qualification for teaching at a university). Students at Lyon follow as a matter of course the laboratory-based *magistère* curriculum. They spend the last of their four years either preparing for a two- or three-year PhD course or in qualifying as fully fledged teachers in higher education (the process known as *Agrégation*. Aubert expects that 9 out of 10 will head towards a PhD.

■ **Internationalization.** Like everybody else in France, Aubert is scheming to do his bit for the integration of Europe. One possibility is to recruit students from elsewhere to Lyon, perhaps as many as 50 each year. (The statutes allow foreign students to attend, and to pay no tuition fees, but they would not be paid or other-

wise dealt with as if they were French civil servants.) News of this scheme advertised on Lyon's research network apparently brought 18 university people from elsewhere in the European Communities and the European Free Trade Area to an enthusiastic meeting on 7 June. Further developments are awaited.

■ **Liberalization.** To guard against the danger that the *normaliens* will be over-narrowly educated, there is a scheme to mix students from the University of Lyon in with them. But there are no plans to follow, for example, the Massachusetts Institute of Technology in introducing liberal studies of some kind to the curriculum.

■ **Research.** In the long run, the success of the Ecole Normale Supérieure de Lyon will hang on the reputation it acquires in research. There are almost 150 researchers already at work, two-thirds of them on the payrolls of either CNRS or INSERM. It is a guiding principle that there should be strong links between the school and the national research organization, with the result that two of the university's six laboratories are joint CNRS-Lyon institutes while the others are less formally linked to CNRS. Aubert is entirely content to think of using laboratories at Grenoble as well as Lyon both as research partners for Lyon's academics and as places at which the *école's* students can follow their *magistère* programmes.

Much thought seems to have gone into the formulation of research programmes which, while having roots in traditional research, are conceptually innovative. For example, the people in *informatique* are concerned with the logic of parallel computers, the physics department with instabilities and order-disorder transitions in liquids.

So has the move indeed confirmed that it is possible to live, and to remain intellectually alive, outside Paris? Almost all the academics at Lyon have moved from Paris, although not from the old campus at Saint Cloud. Interestingly, three people (for this purpose a random sample in that their qualifications were that they speak English) had all used the opportunity of the move to Lyon to change the emphasis of their research. One common goal seems to be the creation of a distinctive line of enquiry, capable of catching national and international attention.

Those who have made the move are youngish people (and Aubert is proud of the average age of the faculty at Lyon). The reason is straightforward: as one explained, "the older people would not move". But even some of those who have done so acknowledge that there are ways in which "Paris is better". What they mean is that the extramural aspects of life in Lyon are not as varied as in the capital. That is perhaps something else on which Aubert should be working. □

What's in a number?

TELL the average academic in Chicago, Manchester, Tokyo (and probably anywhere else outside France) that you teach at the University of Paris and he is likely to say "Ah!, the Sorbonne", perhaps adding that it is "the oldest university in the world, isn't it?". In fact, the Sorbonne ceased to exist in 1790 and was given to the University of Paris in 1808.

In troubled 1968, that was divided into 13 campuses or, more precisely, what is now the Académie de Paris comprises 13 universities — each one numbered. But the numbers have verbal equivalents. A researcher in physics might, for example, give his address as "Paris XI", but say he works at "Orsay". And the rector would call himself "*Professeur en Sorbonne*", not at Université de Paris I.

The confusion is even greater than it first appears. While three campuses might still call themselves "the Sorbonne", being on the original campus in the Latin Quarter, Paris I is known as "Tolbiac", Paris III as "Censier" and only Paris IV, the old faculty of letters and arts, still calls itself "Sorbonne". Meanwhile, the Université Pierre et Marie Curie is always known as Paris VI, while Paris VIII, set up as an experimental university after May 1968, is called "Vincennes", after its original location in the eastern suburbs, but is now at St Denis in the north.

Foreign academics would, however, be right in thinking that the University of Paris is, with Bologna, older than other European universities. It began as a theological school attached to the cathedral of Notre Dame. As the number of tutors

MAGISTERES

Gaining distinction by degrees

If universities can do little to select the students they teach, they can at least seek to distinguish between those who leave with degrees. That is one function of the *magistère* programme, originally conceived by Jean-Pierre Chevènement in the early 1980s as a way of redressing the balance between the *grandes écoles* and the regular universities.

The result is a highly selective beefed-up diploma for university students called the *magistère*. First introduced in 1985 in 70 selected university departments, in a wide range of disciplines, the *magistère* is meant to prepare students for research or industrial careers no less promising than those of engineers from the *grandes écoles*.

Students enter the *magistère* after the first cycle (first two years) of university education, but must have a special mention

grew, so did the variety of disciplines. The whole became known as the university, while tutors within a discipline grouped together as faculties.

The Sorbonne appeared in 1253, as a theological school founded by Robert de

PARIS UNIVERSITY BY NUMBERS

Paris I	Pantheon-Sorbonne	"Tolbiac"
Paris II	(economic law, social sciences)	"Assas"
Paris III	Sorbonne Nouvelle	"Censier"
Paris IV	Sorbonne	—
Paris V	Rene Descartes	—
Paris VI	Pierre et Marie Curie	—
Paris VII	—	"Jussieu"
Paris VIII	Vincennes at Saint-Denis	—
Paris IX	Dauphine	—
Paris X	Nanterre	—
Paris XI	Paris-Sud	"Orsay"
Paris XII	Paris Val de Marne	"Cretail"
Paris XIII	—	"Villetaneuse"

Sorbon, and had a stormy history. It was opposed to the establishment of Jesuit orders in France and sided with the English against Joan of Arc. The Sorbonne was closed by Convention in 1790.

Once integrated into the University of Paris, the "Sorbonne" refused to accept the teaching of new disciplines. Consequently, new universities were founded to teach sciences, humanities and the social sciences. Only after 1968 did universities throughout France become truly multi-disciplinary. Today, in the first major reforms since the 1970s, eight new universities are planned, four in the Paris area.

With the growth of the university system as a whole, the numbers game has spread. In Montpellier, for example, there are "Montpellier I", "II" and "III", the first (teaching arts, literature and the humanities) also known as "Université de Paul Valéry".

Peter Coles

in their diploma (DEUG). Because the *magistère* degree is not national, but awarded by the university, students almost always carry on their regular studies.

In the first year, students carry out supervised research at a state laboratory, one half day each week. In the second, they are working full-time in a research laboratory and, in France, are given priority for grants under the European Commission's Erasmus and Comett programmes to study abroad. Students from the Joseph Fourier University at Lyon last year went to the University of Sussex and others to Philips in Eindhoven, for example.

The ministry of education has no plans to expand the scheme at present; much will no doubt depend on an evaluation of the scheme completed by Dr Guy Aubert.

Peter Coles

GRENOBLE

Disassembling Alpine science city

If France were the Soviet Union, this Alpine city would be named Akademgorodok and everybody would be a little self-conscious about its strategic and economic importance. But the city's ambitions seem to lie somewhere between an upmarket ski resort and a smaller version of Paris (to which every provincial city aspires).

As if in that cause, the mayor of Grenoble, M. Carrignon, has in the past few weeks put the city on the national political map by advising members of the Republican (centrist) party to which he belongs to vote Socialist in the local elections so as to defeat the candidates of the extreme-right National Front. For his pains, Carrignon has been suspended from party membership.

But the nature of the place is apparent from the autoroute from Lyon, just over 100 km away. First, there are traditional conveyor belts carrying limestone from a scar on a miniature alp to the east to the cement factories in the valley of the Isère, then the unmistakable outline of a particle accelerator taking shape at about the point at which the exit signs read "*Polygone scientifique*". The circular hole is, of course, the European Synchrotron, the 6 GeV electron synchrotron designed to be a purpose-built source of X-rays and γ -rays when it is commissioned in 1993.

Grenoble has grown to be the place it is by a sequence of accidents, among which the first was its choice as the site for the basic research laboratory of the Commissariat à l'Energie Atomique (CEA). Another influence is more personal — that of Dr Louis Néel, who was still a member of the physics department at the University of Grenoble when he won a Nobel Prize (in 1970) for the discovery of antiferromagnetism. Néel laid the foundations for tempting to Grenoble the half a dozen CNRS laboratories, mostly centred on condensed-matter physics, that constitute the Polygone.

What more natural than that the city should also be the site of the thermal nuclear reactor which, under the name of Institut Laue-Langevin (ILL) is one of Europe's most prolific sources of thermal neutrons, used mostly for structural studies? The institute is jointly financed by France and West Germany, with smaller contributions from other members of the collaboration. Tucked away in the same *polygone* is a Max-Planck Institute of solid state physics, visible proof of West German commitment to the place.

Guessing at the numbers of scientific and technical people is more difficult at Grenoble than at, say, Novosibirsk, but CNRS is thought to employ about 700 qualified scientists here, CEA perhaps twice as many, while the less permanent populations of ILL and, eventually, of the

European synchrotron laboratory, may account for a further 1,000. Then there is the university. Allowing for technicians and administrative people, direct employment in Grenoble's public laboratories is probably not far short of 15,000, a substantial proportion of the regional population, estimated at about 400,000.

But that, people are quick to say, overlooks the uncounted scientists and engineers working for the small technical enterprises that have sprung up (with the encouragement of the local authorities) at Grenoble, originally as suppliers of goods and services to the public laboratories, but which have now cultivated national and international ambitions. (The continuation of the autoroute from Lyon is plainly marked "Turin", but francophone Switzerland is even closer.)

Grenoble, in other words, is a living proof that life outside Paris is possible, and can even be rewarding. One benefit has been that the laboratory-based culture has been able to forge a strong relationship with the University of Grenoble, to their mutual benefit. CNRS laboratories regularly have academics among their staff members, while researchers teach courses at the university. (The incentive is not monetary, but the simple consideration that teaching experience counts as a plus when promotions are considered within CNRS.) Similarly, academics and people from the various laboratories sit on each others' assessment, promotion and strategic committees.

The academic links with research are plain to see. At the CNRS Laboratoire d'Etudes des Propriétés Electroniques des Solides, graduate students (from other universities as well as Grenoble) outnumber fully fledged researchers (a third of whom are Grenoble academics).

This fruitful symbiosis rests in part on bread-and-butter considerations: academics have access to more sophisticated equipment than university laboratories can usually provide, while laboratories can profit from the flexibility of the funds provided by the university towards the overhead costs of their academics' and their students' research costs.

But there is more to say than that. Dr Guy Aubert, head of the CNRS Service National des Champs Intenses until two years ago says that, in the physical sciences, CNRS offers the only mechanism in France by which the quality of the research people do can be sympathetically and objectively assessed. To link a university with the CNRS system provides it with a yardstick for the measurement of self-improvement and the means thereto.

It goes without saying that the symbiosis provides the laboratories with recruits who can hit the grounds running. Gre-

noble is both the originator and an enthusiastic exponent of the *magistère* programme (see opposite) by which students are introduced to research while still working for their first degrees, partly by being taught by researchers and partly by working in research laboratories or at computer consoles. The *polygone* has provided a sparkling new building in which to house the *magistère* students.

There is a general lesson to be learned from all this: CNRS laboratories can profoundly influence a nearby university. It is not just that CNRS laboratories can provide neighbouring academics with facilities for research that would otherwise be lacking (thereby making the universities attractive to potential teachers), but that they can also hope to mould both the university curriculum and the composition of its faculty.

But the symbiosis requires readiness on both sides. Other CNRS laboratories complain that they must take in graduate students from neighbouring universities over whose curriculum they have had virtually no influence. *Formation*, the academics say, "that's our responsibility". Then the symbiosis cannot work. Nor can it when there is no CNRS or INSERM laboratory within reasonable range.

It is no wonder that Grenoble (with Strasbourg, Orsay and Toulouse) has been singled out as one of the outstanding university centres in France. In reality, there are both the Polytechnic University, with 2,000 students, and the three campuses of the University of Grenoble dealing (by numbers) with science and technology (the Joseph Fourier University), with social sciences and with arts and literature. The city has a student population of 30,000.

Not everything in Grenoble is lovely. The worry about salaries, generally through CNRS, quickly bubbles to the surface. Working researchers say that industrial salaries are generally 50 per cent higher than their own. Most people acknowledge that it is worth paying something for the extra freedom and interest that their laboratories allow. But 50 per cent? Guy Aubert says that even laboratory chiefs are now regularly head-hunted — most expect to get one reasonable offer a year.

Promotion is also a constant worry. Becoming a *directeur de recherche* (a salary grade), requires not just a *concours*, but passing through the eye of a needle which appears ever to shrink in aperture.

Some at Grenoble are also alarmed at the shrinking proportion of CNRS's income available for the direct support of research. This relatively well-heeled group of laboratories is well placed to secure outside contracts, and does well, but is alarmed that external dependence may eventually threaten what it considers to be its core research. □

A European Los Angeles?

MONTPELLIER has all the makings of Los Angeles, but it has a long way to go. For the time being, it differs by a fragment of a substantial mediaeval city wall, the like of which has never been seen in California, while it is still possible to look at clear blue sky. But not for long, if the fastest-growing city in France can keep up the momentum, it may soon have enough road vehicles to generate a pall of smog.

Montpellier also has the distinction of beginning as a university town. There are 200,000 permanent residents, but 50,000 university students who come and go.

Apart from the abundant youthfulness, the city has gone in for three contrasting and inconsistent architectural gimmicks whose like cannot easily be seen elsewhere. First, the old centre of the city has been covered with a sheet of terrazzo as vast as St Peter's Square, from whose edges protrude the upper floors of modern buildings, most of them apparently triangular, which are founded on solid earth. (The centrepiece of the display is a theatre, the Comédie, in which few plays play.)

Second, there is a gigantic air-conditioned bunker a good 300 m long in a distant corner, said to have cost more than \$2,000 million to build. It is a conference centre of the most modern kind, which provides the city with a car park and which is inhabited by the chamber of commerce and industry.

Third, there is the new home of the Conseil Général, the provincial government, constructed to a kind of athenian plan; there are half a dozen post-modern replicas of the portico of the Parthenon (with office suites behind) arranged in a semicircle and separated by a highway and an artificial river from the central office block, in glass and soft brown stone rising to a dozen stories. One Montpellier driver thinks the river a huge joke: "it would take a long time to get to the sea that way".

What is happening is that Montpellier believes it has a chance to become the most important city in the arc of the Mediterranean between Gibraltar and Marseilles. One piquant feature of the development is that the Socialist mayor of the city, Georges Frèche, and the Republican administration of the region, have sunk their political differences in this common cause.

Professor Roger Brunet, a geographer who is the director of the CNRS-financed "public interest group" RECLUS based at Montpellier, explains that there is a "launch-window" within which the city must somehow make its mark. ("RECLUS" stands for Réseau d'Etude des Changements dans les Localisations et des Unités Spatiales.)

The window opened in 1988, when the

national government devolved to the departments certain responsibilities for education, planning and industrial development, and when Spain and Portugal joined the European Communities, enlarging the city's vision to the West. But growth had begun before that, in 1965, when IBM chose Montpellier for a manufacturing plant (now the chief source of employment after the three universities). Legend has it that somebody at IBM drew a line from the company's research laboratory at Nice to its import base at Bordeaux, and found Montpellier roughly half-way between.

With that beginning, Montpellier's ambition is to become a technopolis — one blessed with sun and sea (5 km to the south, past the airport and through a group of small factories). There are also the Cevennes mountains 10 km to the north. The other assets are the universities and the large group of research council laboratories (INRA is especially well established) scattered about the city. The city is also the main base of CIRAD, with responsibility for the cooperative development of tropical agriculture. The city and

NUMERACY

Industrial mathematics booms

INDUSTRY seems to have taken to the heavy-duty computer and the techniques of model-building with the enthusiasm with which the French public has embraced Minitel — the ubiquitous domestic videotext service originally devised as an electronic telephone directory, but now used for gathering general information, train times for example. The result is that industrial researchers have set out deliberately to recruit mathematicians to their cause.

The impetus is twofold. Simulation is often much cheaper and quicker than experimental investigation, vividly apparent in the use of simulation by aeroengine manufacturers in the design of turbine blades (whence, in part, the present concentration of interest in fluid dynamics). The recognition of that truth is not unique to France. But what may be is the recognition by French industry that mathematics is one of the country's strongest suits, and that mathematicians are not always as unwilling to bother with practical problems as their reputation suggests.

But how to capture that resource? Elf's Solaize laboratory believes it has found an effective way of doing this, on behalf of the company's research effort as a whole. J-P. Valentin is a regular mathematician on the laboratory's full-time payroll who is not merely allowed, but required, to spend half his time on academic work.

the region are setting out deliberately to exploit these assets, seeking to attract small and large enterprises in agriculture, pharmaceuticals and *informatique* to science parks physically designed to be contiguous with the laboratories in related fields.

Brunet, whose unit has general interests (it is using its expertise in cartography to compile an atlas of the world as well as of France, and has advanced the doctrine of territoriality as central to the present development of Europe) is a bystander, but an interested one. One of his projects is the conceptual design of a highway from Gibraltar to Constantinople, on which Montpellier would be a crucial staging-post.

So when will the launch-window close? Montpellier seems realistically to appreciate that the world has become competitive. Toulouse, the centre of the aerospace and much of the electronics industry, is just 100 km to the northwest. More irritating, Nîmes, 50 km in the other direction, and best known for its splendid Roman amphitheatre, is nursing ambitions to expand (as, indeed, it is). The window will close only if the plans now hatched fail to materialize. Nobody in Montpellier believes that will happen. □

For the rest, Valentin's job is a mix of persuading researchers in the company's laboratories that many of their problems are often essentially mathematical problems and then of recruiting appropriate outside consultants who can help to solve them. He says that he would be unable to perform the second role if he were not himself a practising mathematician, able to mix with others on equal terms at conferences.

He describes the first function as if it were a kind of psychoanalysis. Most researchers, unaware of recent advances in mathematics, are slow and even unwilling to acknowledge that their problems may be mathematical in character. People used to making measurements with an experimental rig prefer to stick with what they know. He also claims to be a kind of midwife, recognizing that one research group may already have a solution to another's problem.

Valentin cites as an important technique in his business the organization of regular meetings at which Elf-sponsored PhD students (there are 120 of them scattered about the universities) present accounts of their research in the presence of their academic supervisors. These serve, he says, not just as ways of recruiting consultants, but as a powerful means of deepening the company's corporate culture of mathematics. □

NUCLEAR ENERGY

Reactors hit financial trouble

FRANCE's ambitious nuclear programme has wound up in a curious dispute over the ownership of the chief reactor-builder, Framatome, in whose shares its chief rival, the Compagnie Générale d'Electricité (CGE) has acquired a controlling stake. The government is in a quandary over whether to renationalize Framatome.

This is an unexpected but unavoidable outcome of the past two decades, during which France has striven to make its nuclear energy industry second only to that of the United States. But now, with a slump in oil prices and a worldwide wariness after the Chernobyl accident, it has found itself with knowhow nobody wants and more electricity than it needs.

The 53 reactors belonging to the nationalized EDF (Electricité de France) — mostly of the pressurized water type, but including one fast-breeder reactor — supply 75 per cent of the country's 342,000 million kilowatt-hours of electricity production. With very few coal, gas and oil resources, France began strengthening its nuclear capability during the 1970s oil crisis. Successive governments continued the expansion policy, supported by a generally enthusiastic public.

France also has stakes in all aspects of the generation cycle. Cogéma, the industrial subsidiary of the Commissariat à l'Energie Atomique (CEA), the state research agency, has almost cornered the world market in irradiated fuel reprocessing at its La Hague plant on the coast of Normandy, on the English Channel, with a capacity intended to be 1,600 tonnes a year by 1992 and as much as 40,000 tonnes by 2000.

Just as France's own construction programme matured, the Chernobyl accident led other countries to freeze their construction programmes, leaving France's two major manufacturers of nuclear technology, Framatome and CGE, with little hope of critical export orders. The situation was made worse by a saturated home market. In the 1980s, ten new reactors were commissioned to meet an expected demand that never materialized. And a new generation of fast-breeder reactors was planned to follow the experimental 1,200 MW Superphénix plant at Creys-Malville on the Swiss border. But soaring costs, unreliability, cheap uranium and national overproduction led this programme to be shelved.

To add to the slump in enthusiasm for nuclear reactor construction, doubts have now been raised about the safety of existing reactors. Earlier this year, it was revealed that the wrong bolts had been used in maintenance on safety valves at the Gravelines site on the English Channel coast near the Belgian border. A report commissioned by EDF revealed

similar incidents elsewhere and that the risk of a major accident in the next 20 years at one of the French reactors could be much higher than previous estimates. There are six 1,300 MW reactors on the Gravelines site; even the remote threat of an accident has made France's neighbours twitchy.

But the need to replace old reactors, combined with expectations that industrial growth will increase energy demand in the next decade, means that the reactor construction programme is not dead. Only last week, EDF promised that a new 1,400 MW reactor at Civaux would get the go-ahead next year after long delays.

The prospect of renewed growth has put Framatome and CGE at the heart of a political storm. Last month, CGE bought out shares in Framatome held by Dumez, giving it a 52 per cent of the company, a controlling stake. The government feels uncomfortable with its 45 per cent minority share (35 per cent CEA and 10 per cent EDF) and is now wondering whether to intervene by nationalizing Framatome again, so as to gain control over little-valued but potentially critical expertise.

CGE, privatized in 1987, is against such a move and, in any case, nationalization is likely to run up against a ruling of the present government guaranteeing neither privatizations nor nationalizations of industries in the current mandate.

Peter Coles

INDUSTRIAL RESEARCH

Getting back to business

CRUDE oil refiners in France have been driven into a corner — and into research — by the nuclear energy industry, and by the collapse in the market for heating oil. Elf-Aquitaine, which operates three refineries in France, says that the annual throughput of crude has fallen from 100 million tonnes a year to only 80 million tonnes, and demand for heating oil has fallen even more drastically.

The problem is how to win a greater yield of saleable products, notably petrol (gasoline, otherwise *essence*) by the intelligent cracking of crude. The company claims to be a pioneer in the automatic control of refinery operations. Now, at its research laboratory at Solaize, south of Lyon, it has a team of eight people perfecting the operating parameters of catalytic petroleum crackers, believing that understanding will lead to a greater yield of lighter fractions.

The research programme has been a joint effort by Elf and CNRS since 1956. A group of four CNRS researchers have been seconded to the Elf laboratory. When their project finishes two years

Science parks multiply

RESEARCH may not immediately make nations prosperous, but it can do wonders for the prosperity of cities. That seems to be the principle on which several municipal governments are now working, from Lille and Rennes in the north and Lyon in the centre to Montpellier in the southwest.

So science parks are multiplying. At Montpellier, there are no fewer than three, conceptually centred on the city's intellectual assets.

The presence of IBM has stimulated the city, in the 1980s, to persuade small companies in *informatique*, robotics and artificial intelligence to what it calls its Parc du Millénaire.

Agropolis is the name for the similar effort in agronomy and agricultural biotechnology, the core of which is the complex of government laboratories with interests in food and agriculture, between them employing an estimated 1,800 people.

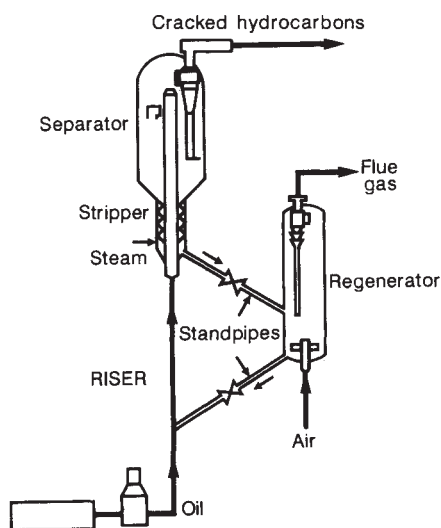
There is also what Montpellier calls its Euromedicine park, to which the city has attracted more than 20 businesses and research laboratories in the fields of pharmaceuticals and medical instrumentation.

What stands out from these endeavours is that the public laboratories are more than merely compliant, they are encouraging. It is public policy not only that France should increasingly rely on technology for its prosperity, but that neighbours with interests in research may be a source of research contracts, perhaps even of better jobs. □

from now, the CNRS people will return to their laboratories not merely with a better appreciation of industrial problems, but with an expertise in the operation of large pilot plants. Nobody seems alarmed that they will also be the better able to advise Elf's competitors.

The problem of optimizing the design and operating parameters of a cat cracker is complex. J.R. Bernard, the Elf researcher who leads the group, likens it to the problems that arise in the design of nuclear fuel rods and the fixing of operating parameters.

The catalysts are zeolites, used in the form of 60- μ m grains which give the material the appearance of a brownish-pink powder. Standard practice entails the use of heavier distillation fraction of the crude to support an upward-moving fluidized bed of catalyst in what is inevitably called a 'riser'. The cracking process appears to require as little as 2 seconds, which is a pointer to the speed at which materials are circulated through the plant. Petroleum products are stripped from the catalyst by steam and the catalyst recycled



Catalytic cracking plant Elf style.

through an air-driven regenerator whose chief function is to remove solid carbon, or 'coke', from the catalyst (see figure).

In the drive for increased gasoline output, schemes for the hydrogenation of heavier crude fractions are being canvassed, further complicating the optimization of cracking plants.

At Solaize, the joint Elf-CNRS team has been forced back to basics, fitting a pilot-plant housed in a hangar-like

building with instruments and using radioactive tracers for tracking the movement of zeolite through its cycle. The objective is to refine a mathematical model of a cracking plant which is being elaborated, with the objective of fixing the parameters of more efficient cracking plants linked with hydrogenation units. The same model will eventually be the basis of programs for the automated control of refinery operations.

Bernard enthuses about the niceties of the operation of standard cracking plants which the study has brought to light. Even questions such as the effect of the riser walls on the movement of zeolite had previously gone unanswered, he says. That there should be friction had been expected, but the velocity profiles across the riser that have now been measured, and the patchiness of zeolite concentration across the riser, were not expected.

Nobody at Solaize is able to say what the economic benefits of the programme will be. Rather, the assumption seems to be that a business which depends on the successful operation of machines such as cat crackers had better understand them in as much detail as it can.

Elf is not alone among French companies in having taken a deliberate decision in the past decade to spend more

of its effort on basic research. Claude Jablon, corporate director of research, says that the company (which differs from more conventional oil companies in its strong interest in chemicals and pharmaceuticals) employs 4,500 people in research laboratories scattered through France, elsewhere in Western Europe and in North America. Of this effort, engaging some 4 per cent of the company's total staff, he reckons that 10 per cent is devoted to projects that will yield benefits only in the long term.

Just where the project to develop better asphalt fits in this is not clear. The company appears to have something of a success with a material called 'Styrelf', selling 300,000 tonnes a year of it. The idea is that hydrocarbon molecules of regular asphalt (non-volatile refinery fractions) have been covalently linked with styrene to form a kind of three-dimensional molecular network in which road metal (graded granite fragments) for highway surfaces can be embedded. Energy is being spent on the materials testing of various compositions of road surfaces, but, in the absence of a full understanding of why highway surfaces wear out or become distorted, faith is required to assure highway authorities that polymeric asphalt will last longer. □

SPACE

Is there profit as well as pride?

OVER the past 25 years, France has emerged as the European leader in space technology, continuing to push forward and take expensive risks while its neighbours got cold feet. This fits with the high profile given to technology.

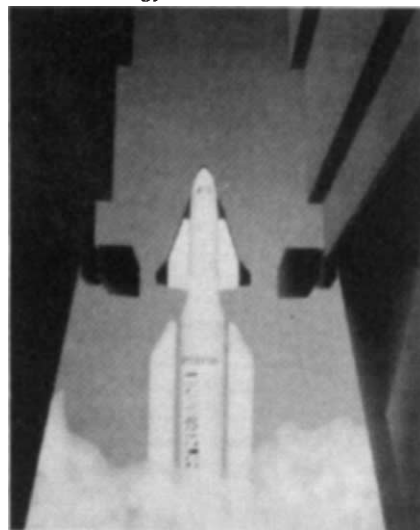
The academic elite graduating from the *grandes écoles* are called 'engineers', and electronic gadgetry sprouts regularly in Paris, from video in the metro stations to cable television. The Minitel telephone-linked interactive videotext system is the most successful in the world and France's high-speed trains (TGV) and modern metro systems are often cited as models elsewhere. High technology is seen as the key to economic survival in the next century.

France has had its own space programme since 1962, when the Centre National d'Etudes Spatiales, the national space agency, was founded. In 1975 it abandoned its own domestic rocket programme to cofound the 13-member European Space Agency (ESA).

Now, France contributes almost a third of ESA's \$1,700 million budget, ahead of Germany and Italy and four times as much as Britain. It is no accident that ESA headquarters are in Paris, that the European commercial launch consortium — Ariane-space with 50 per cent of the world market — is based in France, or that the first

European cosmonaut was a Frenchman.

Always an enthusiastic partner in ESA, France pulled out all the stops at the 1987 ministerial meeting in The Hague, at which the agency's long-term plan was approved. The Hermes space plane developed by CNES was chosen to service the Columbus platform, intended as Europe's contribution to the US Freedom space station, despite criticism from Britain that the technology was out of date and that



Ariane 5 will launch Hermes — both products of French initiatives.

the plane was too small.

But the price of the acceptance of Hermes is that France has to foot 45 per cent of the bill, a cool FF597 million in 1990. Furthermore, Hermes depends on a new generation of heavy-lift launcher, Ariane 5, to get into space and this, too, is 45 per cent French, with costs to France running at FF1,812 million this year.

But while Britain decided that the Hermes and Ariane 5 package was simply too expensive and dropped out, France found it had money to spare to boost its domestic space programme. In the 1990 civil research budget, CNES received FF7,187 million — almost 16 per cent of the entire budget and 11 per cent more than 1989.

Of this, FF352 million will go towards a fourth generation of the SPOT commercial remote-sensing satellite owned by CNES, which will have cost an estimated FF2,500 million by the time it is launched in 1995. Although SPOT loses money, its freely available high-resolution photographs have proved a thorn in the side of superpower defence departments and a way to vaunt France's prowess.

As European space technology has a distinctive French tinge, it is fitting that the new director of ESA should be a Frenchman, Jean-Marie Luton, who came from CNES. And with some tough negotiating ahead as the full costs of Hermes and Ariane 5 emerge, Luton will need all the backing he can get.

Peter Coles

The arid–humid transition in the Sahara and the Sahel during the last deglaciation

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At the time of the Last Glacial Maximum, the Sahara and Sahel regions of North Africa were extremely dry. New records of rainfall show that, during the subsequent deglaciation, the transition from arid to humid conditions in these regions occurred synchronously in two main steps. Comparison with other records of palaeoclimate in Europe and the North Atlantic Ocean shows that certain common factors controlled changes in ocean and atmosphere dynamics during the deglaciation.

THE structure and timing of the last deglaciation is now well documented by numerous oceanic records and continental sequences between middle and high latitudes¹. The last glacial–interglacial transition is not a simple linear response to astronomical forcing, and the two-step deglaciation model is now supported by detailed evidence^{2–7}. Results of general circulation models suggest that climate change in the regions around the North Atlantic Ocean has been influenced greatly by changes in the northern Atlantic Ocean dynamics associated with the meltwater history of the Laurentide Ice Sheet. The effects of cold melt water may explain much of the nonlinear response of climate to astronomical forcing at high latitudes, but they do not provide a complete explanation for the sequence of change observed over North Africa⁸.

In the Sahara and the Sahel, the Last Glacial Maximum (LGM) coincides with an episode of extreme aridity, whereas humid conditions prevailed during the early and mid-Holocene⁹. From simulated hydrological budgets, precipitation over the Sahel at 9 kyr BP (before present) was estimated at 125–130% in comparison with today¹⁰. The last deglaciation period thus corresponds to a major arid-to-humid transition (AHT).

Sediment cores of several tropical lakes span this transition⁹. In western sub-Saharan Africa (~5–15° N) and in the Tibesti mountains, the return of humid conditions before 12 kyr is well known from palaeolake and river-terrace studies^{11–16}, detailed palynological offshore profiles¹⁷, and increases in the discharge of large rivers to the ocean¹⁸. A sudden drop in the level of several tropical lakes around 11–10 kyr has been tentatively linked to changes in the ocean circulation¹⁹. Large inputs of melt water from the Laurentide Ice Sheet in the northern North Atlantic would have reduced the North Atlantic Deep Water (NADW) formation during the Younger Dryas event²⁰, slowing down the North Atlantic's conveyor-belt circulation and inducing anomalies of sea surface temperature (SST)^{19,21}. A colder North Atlantic may have stabilized the lower atmosphere and helped block the advection of moisture over North Africa⁸ and may be responsible for a weaker monsoon rainfall over the northern tropics¹⁹. But few results ascertained from African lands provide a sufficiently defined chronology of the late Glacial to allow for high-resolution correlations with the oceans. Also, very little is known north of 15° N before 10–9 kyr. Because there are no continuous records from the early Holocene in the northern Sahara, the concept of a progressive reinstatement of

a humid climate between the Equator and the Maghreb from 13 to 6 kyr has been advanced^{22,23}. This hypothesis was followed by several authors^{24,25}, and developed in terms of general atmospheric circulation^{23,26}. We show here that the re-establishment of wet conditions occurred before 12 kyr over both the northern and the southern margins of the Sahara, and that the climate evolution of the Sahel has been more complex than previously thought.

Although local hydrological factors may largely influence their water level and water chemistry^{27,28}, groundwater-fed palaeolakes of the Sahara and the Sahel can be very sensitive climate indicators provided that: (1) the extent of the recharge zone of the aquifer is relatively restricted to allow climate reconstruction at a local scale; (2) the response time to rainfall events is negligible; (3) the chemical and, in favourable cases, isotope characteristics of the ground water are known; (4) variations in hydrological conditions are not masked by evaporite dissolution; and (5) the hydrology is not influenced by sea level fluctuations. These conditions are met in two closed depressions selected from numerous sites studied in the PALHYDAF project^{14,29}, in the northern Sahara (Site 1, Sebkhla Mellala, Algeria) and in the Sahel (Site 2, playa of Bougdouma, Niger), respectively (Fig. 1). Cores from the centre of these depressions provide continuous profiles from the late Glacial to the Holocene through sediments of aquatic origin (Fig. 1).

Crucial problems raised in ¹⁴C dating in such environments, especially ageing by groundwater supplies and recrystallization due to water-table fluctuations, have been thoroughly discussed^{15,29,30}. For carbonate dating, authigeny and the absence of recrystallization were verified by scanning electron microscopy (SEM) and the authigenic fraction (generally <80 µm) was separated by sieving. The ¹⁴C dates (conventional and accelerator mass spectrometry (AMS), uncorrected for secular variations) are regarded as valid when the ¹³C content indicates that the total dissolved inorganic carbon (TDIC) of the water body was in (or close to) equilibrium with the atmospheric CO₂. In a given section, interpolated and extrapolated ages were calculated in intervals of similar lithofacies.

Three types of palaeohydrological indicators are considered: lithofacies and mineralogy, stable isotopes on carbonates, and biological remains. The stable isotope (¹³C, ¹⁸O) contents on authigenic inorganic carbonates are normalized with respect to calcite using the proportions, determined by X-ray diffractometry (XRD), of aragonite and dolomite and applying the proper isotope fractionation factors³¹. Carbonates from temperate lakes with very high turnover rates may reveal variations in the heavy isotope content of rains that are temperature-dependent^{32–35}. In seasonally dry climates, the enrichment of the groundwater supply in heavy isotopes by evaporation is the major factor controlling the ¹⁸O content³⁶. In both cases, the crystallization temperature is not an important factor because the temperature dependence $\Delta\delta^{18}\text{O}_{\text{carb}}/\Delta T$ is much smaller than the variation in $\Delta\delta^{18}\text{O}_{\text{carb}}/\Delta\delta^{18}\text{O}_{\text{water}}$. In most North African lakes, the ¹⁸O content of carbonates must be regarded as an indicator of the ratio of groundwater input to evaporation. The ¹³C content gives further information on environmental conditions^{15,28,30} such as water mixing, residence time and the intensity

of biogenic production. Among the biological remains, attention is given to diatoms found in the Sahel sediments which allow chemical variables to be inferred from stratigraphic profiles by using calibration functions established from 200 modern African samples (refs 37, 38 and F.G., unpublished data).

Northern Sahara records: Sebkhia Mellala, Algeria

Site 1 (32°11' N, 5°12' E, ~128 m.a.s.l. (metres above sea level); Fig. 1) is a closed depression, wind deflated through Cretaceous and Tertiary limestones and sandstones. It is hyperarid, with rare precipitation of northern origin (mean rainfall 42 mm yr⁻¹; mean evaporation 2,000 mm yr⁻¹)³⁹. The basin, dry today, is a discharge zone of the large confined aquifer of the 'Continental Intercalaire' which has its source mainly in the M'zab heights ~150 km to the west, but also in the Saharan Atlas mountains (Fig. 1)^{40,41}. This deep (~1,000 m) aquifer supplies the local unconfined aquifer (water table at ~-6 m) through upward leakage of fossil water⁴⁰. The basin has thus registered, with no lag, changes in precipitation in the recharge zones of the aquifer of the 'Continental Intercalaire'. The ground water, of Na⁺-Mg²⁺ or Na⁺-Ca²⁺ and SO₄²⁻-Cl⁻ type⁴¹, is slightly saline (2.5–3.5‰). The stable isotope contents are -8.8‰ relative to standard mean ocean water (SMOW) for ¹⁸O of the water and -11.5 to -9.1‰ relative to the PDB standard for ¹³C of the TDIC. The ¹⁴C content of the TDIC is below the detection limit^{40,41}. The dimension of the aquifer suggests that these values apply to the Holocene.

The depression is filled by 8 m of sebkha and lake sediments (Fig. 1) that lie on the bedrock. Detailed results on the sedimentology, mineralogy, stable isotopes on inorganic and biogenic carbonates, element geochemistry of ostracod valves, and

palaeobiology are discussed elsewhere^{42,43}. The mineralogy and stable isotope variations are shown in Fig. 2. Radiocarbon ages on carbonates are accompanied by ¹³C values > -4‰, far above that of the TDIC of the aquifer. Changes in ¹⁸O content (-7.29 to +4.83‰) indicate large fluctuations in the ratio of groundwater influx to evaporation.

An arid climate is first registered (~15 kyr BP) by the dominance of aeolian sand, in association with gypsum which indicates a discrete and probably continuous discharge of SO₄²⁻-rich ground water. The overlying sediments are mainly composed of chemically precipitated minerals. The absence of detrital material indicates that the supply of local runoff is negligible, and suggests that the dunes of the Great Eastern Erg (Fig. 1) were colonized by vegetation. From 14.5 to 11 kyr, gypsum precipitation predominated in a saline water body, although minor amounts of authigenic carbonates allow AMS dating and stable isotope analyses. The filling of the depression at ~14.5 kyr, is recorded by a drastic drop in ^δ¹⁸O (+0.31 to -7.21‰) and by high values of the ratio of groundwater input to evaporation at 12.8–12.7 and at ~12 kyr. The interval 10.6–9.3 kyr contains mostly gypsum and dolomite. SEM observations show that dolomite crystals are idiomorphic, and thus authigenic. The primary or early diagenetic dolomite represents the thermodynamically stable carbonate species in the Sebkhia Mellala deposits after calcium removal through gypsum precipitation has increased the Mg/Ca initial ratio of 0.7 (ref. 41) in the highly concentrated residual solutions⁴⁴. This disordered (Mg₄₄, Ca₅₆) dolomite is thus considered to be a post-gypsum evaporite. The interval 10.6–10.3 kyr has the highest ^δ¹⁸O values (+1.72 to +4.83‰) in the whole section, and is considered to be the period of maximum local aridity. Arid conditions ended suddenly after

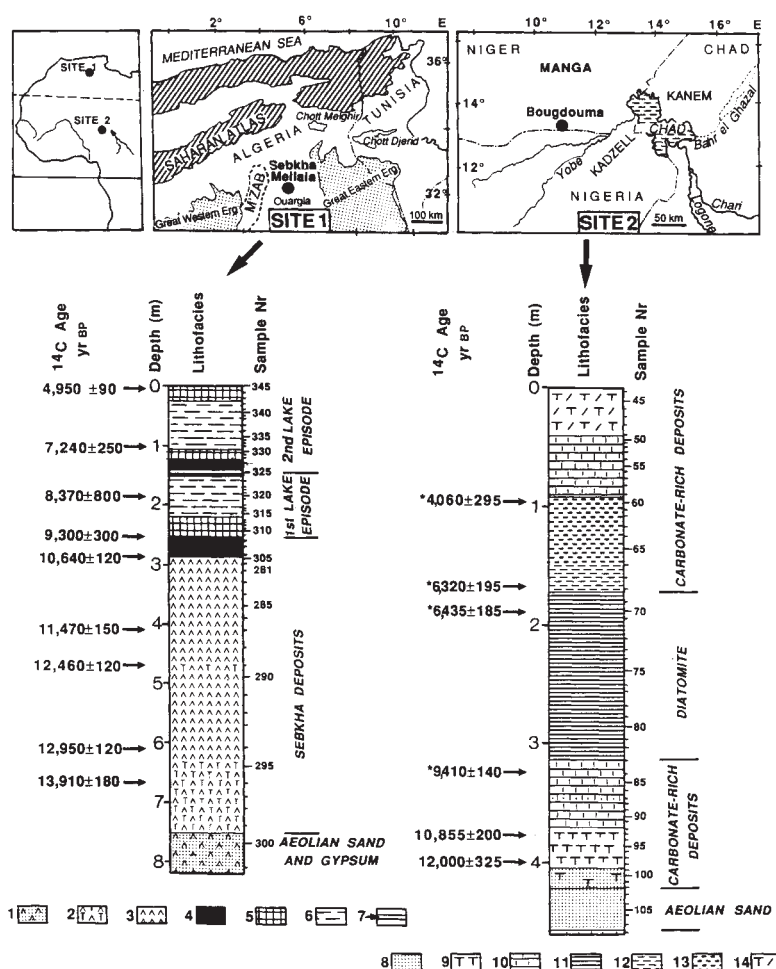


FIG. 1 Location map of the sites and simplified stratigraphy of the cores. Asterisks indicate radiocarbon ages of organic matter, others are ages of authigenic inorganic carbonate. Lithofacies: 1, aeolian sand and gypsum; 2, massive carbonate-rich gypsum deposit; 3, massive carbonate-poor gypsum deposit; 4, laminated gypsum and carbonates; 5, laminated carbonates free of mollusc shells; 6, alternation of shell beds and of carbonate and gypsum layers; 7, palaeosol; 8, aeolian sand; 9, sandy carbonates; 10, diatomaceous carbonates; 11, diatomite-free carbonate; 12, calcareous clay; 13, calcareous sand; 14, carbonates and evaporites.

~9.3 kyr allowing the establishment of a lake, which was maintained until ~7.5 kyr. The humid episode (9.3–7.5 kyr) is registered by an abrupt decrease in $\delta^{18}\text{O}$ (+4.51 to -4.76‰), abrupt mineralogical changes and by the appearance of biological remains. Gypsum is still present, but the dilution of the solutions is indicated by the precipitation of Mg-calcite (associated with aragonite) and of calcite, which successively predominate in the sediments. The biological community reflects a saline shallow, but permanent, water body. No diatoms were found, possibly in response to secondary dissolution. The co-occurrence of freshwater organisms (such as the gastropod *Hydrobia* sp.) and of species able to tolerate marine-like conditions (such as the bivalve *Cerastoderma glaucum*, the foraminifera *Ammonia beccarii*, *Triloculina* sp.) or hypersaline conditions (such as the gastropod *Potamides conicus*, the ostracod *Cyprideis* gr. *torosa*, or the charophyte *Lamprothamnium* cf. *papulosum*) indicates large short-term fluctuations in salinity, a common character of Holocene lakes of the northern Sahara^{27,28}. The lake dried up at ~7.5 kyr, as recorded by a palaeosol bracketed by dolomite-rich deposits, before the commencement of a new cycle of carbonate sedimentation dated from ~7.2 to 4.9 kyr. The top of the sequence is wind deflated.

We therefore identify two major steps in the transition from arid to humid conditions at ~14.5–12 (AHT1a) and 9.3–8.4 (AHT1b) kyr, the first one corresponding to several dry-wet oscillations. A third, smaller, step occurred at 7.5–7.2 kyr (AHT1c).

The Sahel records: Bougdouma, Niger

Site 2 (13°19' N, 11°40' E, 337 m.a.s.l.; Fig. 1) lies in the Sahelian zone where most of the precipitation is due to summer squalls (75%) and monsoon rainfall⁴⁵ (mean rainfall ~250 mm yr; mean evaporation 2,000 mm yr⁻¹ (ref. 46)). The Manga plateau (Fig. 1) is the eroded surface of an old erg, probably of early Pleistocene age, and lies between ~400 (northwest) and 300 (southeast) m.a.s.l. over 120 km. The low gradient and the high vertical permeability of the aeolian sand do not allow for the development of a surface-drainage network. The unconfined aquifer is exclusively recharged by local rainfall⁴⁷, and crops out in numerous closed interdunal depressions. This was also the case during the late Quaternary, contrary to previous opinion⁴⁸ and the results of a related climate simulation⁴⁹, according to which the plateau was submerged by Lake Chad (present altitude 282 m.a.s.l.)^{15,50}. Presently, the deepest depressions are occupied by small water bodies ranging from freshwater to hyperalkaline swamps, or playas with trona crusts (such as Site 2). In January 1989, ground water ($\text{Na}^+ - \text{HCO}_3^-$ type, conductivity = 5,800 $\mu\text{S cm}^{-1}$, pH 8.41, $\delta^{18}\text{O} = -4.01\text{‰}$ relative to SMOW, $\delta^{13}\text{C} \approx 0.0\text{‰}$ relative to PDB) was found 2.80 m below the surface.

The late Glacial climate evolution is deduced from a 4.58-m core (Fig. 1) whose detailed analyses are given elsewhere⁵¹. Figure 3 shows the stable isotope and diatom data. The diatom flora, of fresh to mesosaline water of the $\text{Na}^+ - \text{CO}_3^{2-}$ type, from a neighbouring core have been previously discussed⁵². Sudden peaks of the planktonic form *Cyclotella stelligera* var. *glomerata* reflect abrupt influxes of dilute ground water⁵². High absolute diatom contents coincide with high percentages of freshwater planktonic species (mainly *Melosira ambigua*, *M. granulata* and varieties), and indicate stages of relatively deep and dilute waters. The diatom influx was lower during phases of shallow saline waters. Very low diatom contents associated with bad frustule preservation (between ~12 and 11 kyr, for example) are probably the result of partial dissolution under fluctuating, ephemeral saline conditions⁵².

At the base of the core, a calcareous diatomite could not be reliably dated, even by AMS, because of obvious traces of aquifer diagenesis on the carbonates, and despite the presence of a few millimetre-sized charcoal which are highly vulnerable to impregnation by hydrosoluble organic matter⁵¹. An episode

more arid than today is registered by an overlying pure aeolian sand.

A sudden groundwater influx occurred before 12 kyr, and led to shallow saline conditions, with aeolian sand still predominant in the sediments. A second isotopic dilution is registered at ~12 kyr, and coincides with the base of a calcite-rich (15–50% of bulk sediment⁵¹) sedimentary episode. A water body, possibly ephemeral as suggested by the presence of the ostracod *Heterocypris salinus* and the bad preservation of diatom valves, persisted up to ~11 kyr under highly evaporative conditions. A new rise in the aquifer is recorded at 10.9–10.6 kyr. The isotope concentration then increased and reached the maximum value observed in the section at ~10.3 kyr. Diatoms show that generally dilute open-water conditions persisted up to that date, however, probably because of seepage of heavy solutions through the permeable lake bottom which may have regulated the salinity, as in the case of Lake Chad today^{46,53}. At ~10 kyr, ~300 years later than the maximum isotope concentration, diatoms indicate a brief episode of very shallow saline-alkaline conditions. This is attributed to the leaching, by local rainfall or groundwater influx, of a highly soluble Na^+ carbonate crust deposited during a short but marked dry phase between ~10.3

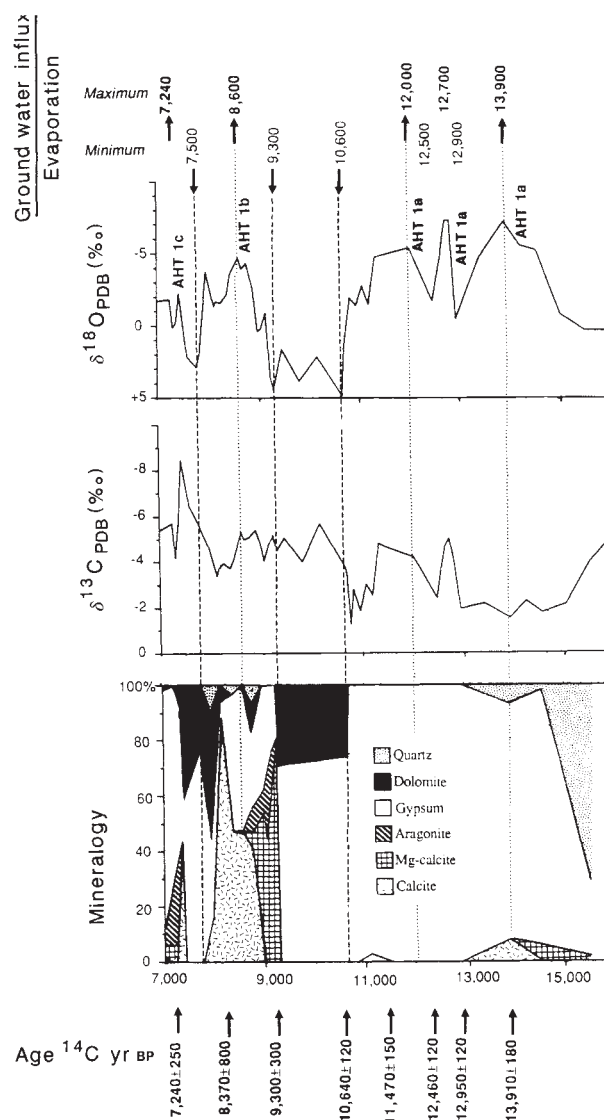


FIG. 2 Palaeohydrological indicators plotted against ^{14}C ages (16–7 kyr BP) for Site 1 (Sebkha Mellala, Algeria). Bottom, Mineralogy (XRD peak areas) of bulk sediment (biological remains excluded). Middle and top, Stable isotope contents of inorganic carbonates (fraction <80 μm) normalized to calcite⁵¹ ($\delta^{13}\text{C}(\text{‰}) = [(^{13}\text{C}/^{12}\text{C})_{\text{sample}} / (^{13}\text{C}/^{12}\text{C})_{\text{standard}}] - 1$; $\delta^{18}\text{O}$ defined similarly). Arrows at the top mark the direction of the major hydrological events.

and 10 kyr. From ~9.7 kyr, the establishment of an early Holocene freshwater lake is registered first by a sharp peak of *Cyclotella stelligera* var. *glomerata*, increased diatom content and salt dilution, and then by a sudden stable isotope decrease. The hydrological turnover peaked at ~9.2 kyr when the calcite sedimentation was replaced by the accumulation of a diatomite almost free of carbonate. The freshwater lake persisted until ~8–7 kyr when the salinity began to fluctuate. Calcite reappears in the sediments at ~6.4 kyr. The top of the sequence records several hydrological fluctuations, including a sudden rise of the aquifer at ~4 kyr.

During the last deglaciation, two major phases of hydrological changes, ~12 kyr (AHT1a) and ~9.7–9.2 kyr (AHT1b), are recorded in the Bougdouma core, bracketing a period of generally high evaporative conditions. During the Younger Dryas, however, there was a brief but clear humid phase dated from

~10.7–10.3 kyr. This is also recorded in individual interdunal depressions along the northwestern margin of Lake Chad^{15,52} and was of regional extent.

Timing of the major climate events

The hydrological settings of our selected sites makes their hydrology and sedimentology dependent on the local rainfall. The two lacustrine records illustrate the extreme rapidity of the climate fluctuations that occurred over the Sahara and the Sahel during the last major arid-humid transition. The shifts from arid to humid conditions occurred in a few centuries, possibly in a few decades. Abrupt climate changes occurred at both sites at >12 (AHT1a) and at ~9.3 (AHT1b) kyr, with a reversal (H-A) centred on 10.5–10 kyr (Fig. 4). Both the northern and southern margins of the desert have thus experienced periods of excess input before 12 kyr, and probably since ~14 kyr. Our

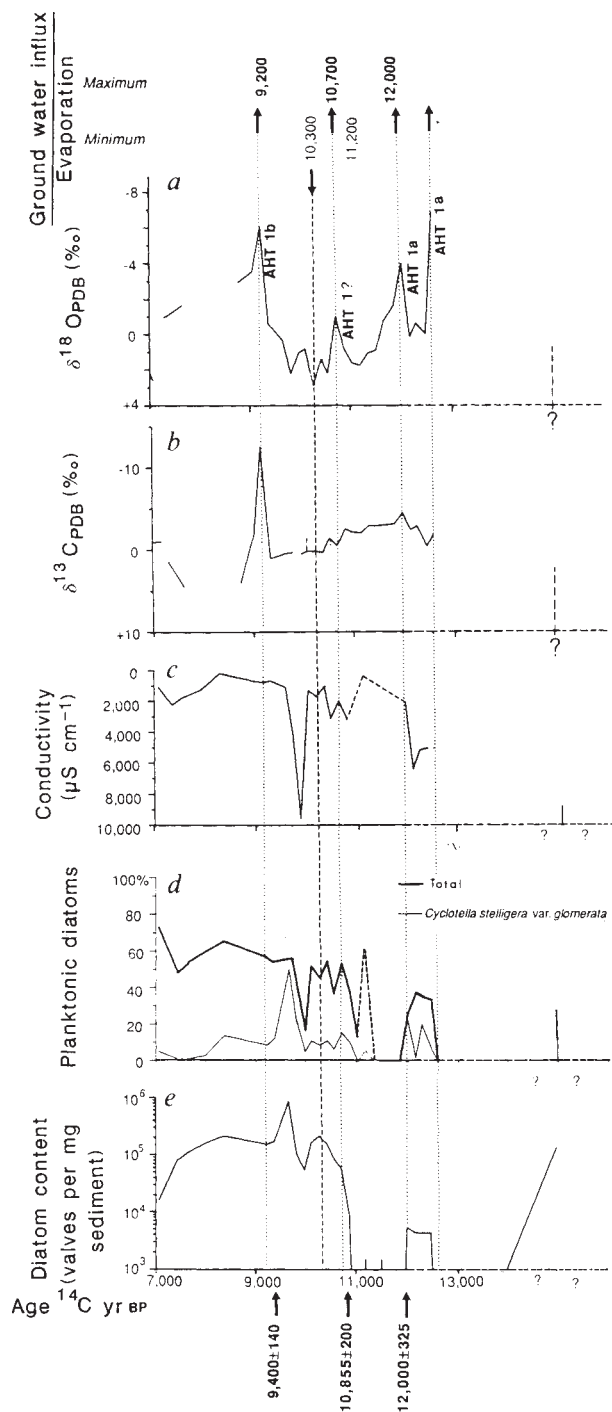


FIG. 3 Palaeohydrological indicators plotted against ^{14}C ages (16–7 kyr BP) for Site 2 (Bougdouma, South Niger). *a, b*, Stable isotope contents or inorganic carbonates (fraction <80 μm). *c*, Diatom-inferred water conductivity based on transfer function (refs 37, 38, and F.G. unpublished data). *d*, Relative percentages of planktonic diatoms. *e*, Sediment diatom content. The results in *c* and *d* are uncertain between 12 and 11 kyr owing to partial selective dissolution. Arrows at the top mark the direction of the major hydrological events.

data agree with previous data documenting the presence of early Holocene lakes in the northern Sahara^{27,28,54}, and do not support the hypothesis of a progressive re-establishment of wet conditions from the Equator to the Maghreb between 13 and 6 kyr^{22,23}.

Relationships to global climate change

Although stratigraphic uncertainties still exist, a comparison between our African sequences and the Atlantic and European records (Fig. 4) suggests that common factors have controlled the major changes in the ocean and atmosphere dynamics at low and high latitudes during the last deglaciation. The principal steps of the arid-humid transition, AHT1a and AHT1b (Figs. 4c–e), seem to have occurred at the same time as two intervals of rapid sea level rise deduced from coral-reef drilling and described as meltwater pulses 1A (12 kyr) and 1B (9.5 kyr)⁶ (Fig. 4h). They coincide with the latter part of Termination 1A, and with Termination 1B as defined from deep-sea ^{18}O records^{2–5} (Fig. 4f, g). Our chronology is also consistent with changes in the mean annual air temperature in Britain (reconstructed using

beetle remains) which show two abrupt rises at ~ 13 –12.2 kyr and ~ 10.2 –9 kyr⁵⁵ (Fig. 4b), and with the European subdivisions based on cold-warm oscillations^{56,57,34,35} (Fig. 4a). In both African sites, the phase of maximum aridity falls in the cold European Younger Dryas chronozones.

In the northern Sahara, the beginning of the first step (AHT1a) at ~ 14.5 kyr corresponds to the first signal of Termination 1A in the North Atlantic^{2,20} and to the first appearances of hydrothermophilous species in the pollen records from southwestern Europe⁵⁸. The phase of maximum aridity started at ~ 10.6 kyr during the first part of the European Younger Dryas, and ended at ~ 9.3 kyr when warm conditions were fully re-established in Europe (Fig. 4b, c). These changes in rainfall, of Mediterranean or Atlantic origin, may have also influenced the vegetation of southwestern Europe.

In the Sahel, the interval 12–10 kyr corresponds with generally high evaporative conditions (Fig. 4d). The maximum aridity recorded at Site 2 between 10.3 and 10 kyr seems to match a maximum in the ^{18}O content of foraminifera and a minimum

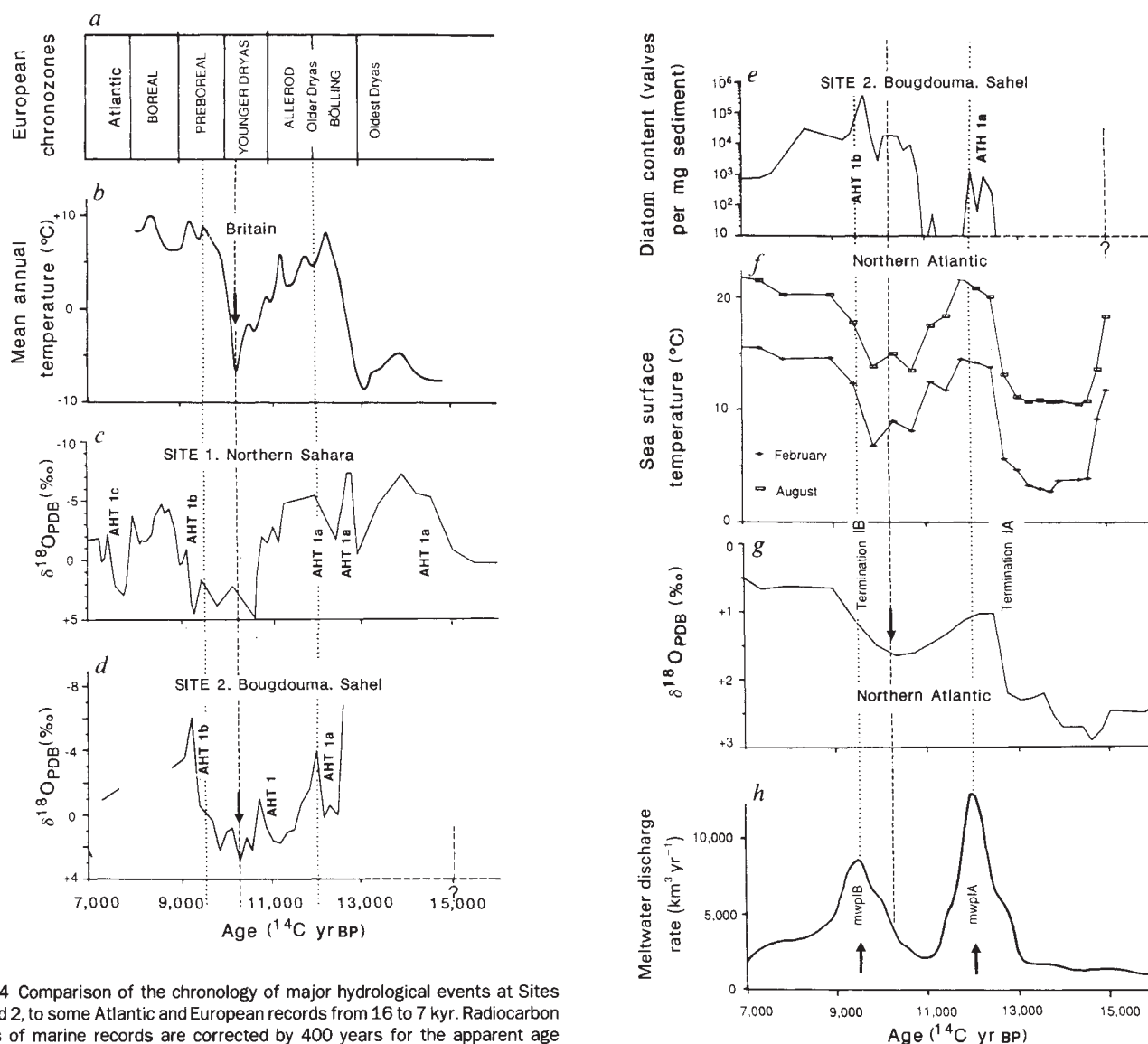


FIG. 4 Comparison of the chronology of major hydrological events at Sites 1 and 2, to some Atlantic and European records from 16 to 7 kyr. Radiocarbon ages of marine records are corrected by 400 years for the apparent age of sea water. a, European chronology^{56,57}. b, The mean annual air temperature in Britain estimated from beetle remains⁵⁵. c, Oxygen isotope profile of inorganic carbonate at Site 1, as an indicator of the ratio of groundwater influx to evaporation. d, Oxygen isotope profile of inorganic carbonate at Site 2, as an indicator of the ratio of groundwater influx to evaporation. e, Sediment content in the freshwater planktonic diatom *Cyclotella stelligera* var. *glomerata*, as an indicator of sudden rises of the aquifer⁵². f, The record

of February and August sea surface temperature from the AMS ^{14}C -dated core SU81-18 located offshore of Portugal based on a palaeontological transfer function (ref. 4, and L. D. Labeyrie, personal communication). g, Oxygen isotope record of *G. bulloides* from core SU81-18 located offshore of Portugal⁴. h, The rate of the glacial melt water discharge calculated from Barbados sea level curve⁶. mwp = melt water pulse.

in sea surface temperature in the ocean core SU81-18⁴, and the minimum mean air temperature in Britain⁵⁵ (Figs. 4b, d, g). In contrast with Site 1, however, sudden reversals are observed at Site 2, as in neighbouring sites^{15,52}, during the interval 12–10 kyr. A humid episode, from ~10.7 to 10.3 kyr, started in phase with the abrupt termination of the Younger Dryas recorded in Greenland ice cores⁵⁹. Modern rainfall on the Sahel is statistically correlated with global SST anomaly patterns⁶⁰. In the North Atlantic (core SU81-18) (ref. 4 and L. D. Labeyrie, personnel communication), the general drop in SST between 12 and 10 kyr is not monotonic (Fig. 4f). An increase of August SST observed from 10.7 to 10.3 kyr might be associated with the synchronous Sahelian humid phase.

Discussion

Major climate changes in North Africa during the last deglaciation seem to be in good chronological agreement with events deduced from deep-sea sediments, ice cores and European climatology. This indicates that the North African climate is largely subject to global (oceanic) control. The effects of cold Laurentide Ice Sheet melt water on the North Atlantic dynamics^{8,20,21} have been invoked to explain the reduction in monsoon rainfall on the Sahel during the Younger Dryas¹⁹. But this does not account for the hydrological evolution at Site 1 because it is supplied by precipitation on the M'zab and on the Saharan Atlas Mountains, and is therefore difficult to attribute

to a monsoonal climate. Nor does it explain the brief, but well defined, humid phase from ~10.7 to 10.3 kyr at 13–15° N, which shows that the concept of a unique recession synchronous with the Younger Dryas cannot be applied to the tropical latitudes of the Sahel. Other minor factors and feedbacks have to be investigated to explain the abrupt climate changes in this region between 11 and 10 kyr. Furthermore, the apparently variable timing of these events calls into question the notion that the Younger Dryas was a global event.

The principal factor inducing rainfall changes over Saharan and sub-Saharan Africa seems to have been the evaporative power of the atmosphere over the major production zones of air moisture. The atmospheric vapour made available by the global warming was distributed over Africa in response to several mechanisms: monsoon, squall lines, westerlies and Mediterranean depressions. We are now investigating the efficiency of air conveyors capable of carrying the vapour masses onto the African continent, especially in the central Sahara where the relative contributions of these effects may have varied during the year. Detailed surveys of laminated sediments are necessary to refine the seasonal variations for coupled atmosphere–ocean general circulation models. Such investigations are expected to reveal whether the different latitude belts of the Sahara and the Sahel could have been influenced by several distinct rainfall seasons during each year in the humid periods recorded by lacustrine events. □

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Evidence for interaction of different eukaryotic transcriptional activators with distinct cellular targets

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The adenovirus E1a protein (E1a), a potent transcription activator, contains a transcriptional activating region. Compared with previously described cellular and viral activators, E1a's activating region has unusual structural properties. It seems that E1a's activating region interacts with a cellular target not required for the function of transcriptional activators with 'acidic' activating regions. By contrast, the target of an acidic activating region is required both by acidic activators and by E1a. It is proposed that the cellular target of E1a's activating region is an 'adaptor' that allows E1a to interact with the basic transcriptional machinery.

THE 289-amino-acid E1a protein is required for the efficient expression of early viral genes¹⁻³. Recent experiments provide evidence that E1a functions at the promoter and has the two essential properties of typical cellular activators: amino acids 121-223 contain both a promoter-binding region that directs E1a to its target promoters and a transcription activating region⁴.

The transcription activating region of E1a⁴ fused to the DNA-binding domain of GAL4 (amino acids 1-147)⁵ increases transcription 200-fold from a core promoter bearing GAL4 binding sites. In contrast, this GAL4-E1a fusion protein does not activate transcription from a core promoter lacking GAL4 sites. The activating region revealed by these experiments is also required by the full-length E1a protein to activate an early viral promoter. Mutations that disrupt E1a's activating region can be rescued by the addition of a heterologous acidic activating region. Thus, E1a's activating region satisfies all the criteria used to define the activating regions of typical cellular activators.

Activating regions of several transcriptional activators have been mapped and grouped loosely into classes on the basis of their amino acid compositions. Activating regions defined to date are rich in acidic residues (GAL4, GCN4, VP16), glutamines (Sp1) or prolines (CTF/NF-1) (see ref. 6 for a review).

The acidic activating regions have been characterized most extensively. Studies of intact GAL4 and GCN4 show that the strength of an acidic activating region decreases only gradually upon deletion⁷⁻⁹. Analysis of the weak activating region of GAL4, region I, and activating regions generated at random from fragments of *Escherichia coli* DNA have verified the importance of acidic residues¹⁰⁻¹³ but, in general, single amino-acid substitutions have little effect. These results suggest that activating regions are not highly structured, although some elements of structure are important for activity^{10,11,14}.

Although different acidic activating regions share no common sequence, they can substitute for one another and probably have

a common cellular target¹⁴. Overexpression of an acidic activating region, for example, can inhibit the function of another acidic activator^{11,15,16}. This inhibition, termed 'squenching', is thought to result from titration of the cellular target of the activating region. Accordingly, stronger activators inhibit (squench) more effectively than weaker ones¹¹. Overexpression of an intact activator can also have an inhibitory effect as a result of 'self-squenching'¹⁴.

Here we present the results of mutational studies of E1a's activating region, revealing its unusual structural features. We also present squenching experiments indicating that the activating region of E1a interacts with a cellular target different from that of acidic activating regions.

Activating region of E1a

Boundaries. Our previous study showed that sequences of E1a between amino acids 139-223 comprise an activation region⁴. To define the boundaries of this region, we constructed derivatives of the previously described GAL4-E1a fusion protein which consists of amino acids 121-223 of E1a⁴. Figure 1 shows that the N-terminus of the activating region lies between amino acids 140 and 142: progressive N-terminal deletion to amino acid 140 resulted in a modest reduction in activity relative to intact GAL4-E1a. A drastic decrease in activity was observed on further deletion to amino acid 143. Activity was completely eliminated by deletion to amino acid 147. Site-directed mutagenesis of the two acidic residues at positions 140 and 141 shows that these positions are not critical for activation by E1a (see below). Thus, the N-terminal boundary of the activating region of E1a is located between amino acids 141 and 142.

Figure 1 also shows that the C-terminus of the activating region lies between amino acids 178 and 173: progressive C-terminal deletion to amino acid 178 did not decrease activity, whereas further deletion to amino acid 172 completely eliminated activity. We conclude that the core of the activating region has discrete boundaries and lies within E1a amino acids 141 and 178.

Sequences required for activation. The boundaries of the activating region indicate that it is contained within E1a region 3, a stretch of amino acids highly conserved among different adenoviruses¹⁷ (Fig. 2a). To confirm the importance of these conserved amino acids for the function of the activating region, we constructed GAL4-E1a derivatives that contain single or multiple amino-acid substitutions. Figure 2b shows that with one exception (substitution of an arginine for the aspartic acid at position 168) mutation of one or more of the conserved amino acids disrupted the function of the activating region. With one exception (substitution of a cysteine for the tyrosine at position 159), mutations of the non-conserved amino acids had no effect.

Amino acids of all classes are conserved (Fig. 2a). Of the conserved amino acids, three are acidic, three are basic, and eighteen are uncharged. Mutational analysis (Fig. 2b) indicated that acidic, basic and neutral amino acids all contribute to form the E1a activating region. For example, basic amino acids at positions 160, 161 and 177, and neutral amino acids at positions 151, 154, 157, 165, 169, 170, 171 and 174 are all important for activity.

It was of particular interest to determine whether acidity is a

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determinant of the activating region of E1a. Substitution of acidic residues at positions 140 and 141 had little or no effect on activity. Changing the aspartic acid at position 168 to a basic residue did not substantially reduce activation. Substitution of a glycine for a conserved glutamic acid residue at position 148 (conserved in five of the six adenoviruses) reduced activity fivefold, whereas substitution of an alanine for the invariant aspartic acid at position 145 (conserved in all six adenoviruses) decreased activation fortyfold. Substitution of a glutamic acid for this aspartic acid reduced activity threefold, indicating that the role of this amino acid is not to provide negative charge *per se*. In summary, although at least one acidic residue (aspartic acid 145) is essential, net negative charge is not a determinant of E1a's activating region.

Four of the conserved amino acids within the activating region are cysteines. These cysteines are the ligands of a metal binding site ('Zn finger') that is required for function of the E1a protein¹⁸. We find that these cysteines are essential for the function of E1a's activating region: changing one, two, or all four cysteines to alanines eliminated activity (Fig. 2b). As model studies have shown that the cysteine residues of Zn fingers are required for metal binding^{19,20}, we conclude that the binding of a metal ion is essential to the formation of the activating region.

Representative activating region mutants were tested for the stability of their protein products; each was expressed comparably to GAL4-E1a (Fig. 2c and data not shown). The small variations observed (less than threefold) cannot account for the large differences in activation. From the sum of these results we conclude that the E1a activating region is not acidic, is highly structured, and includes an essential metal-binding site.

Cellular target of the activating region of E1a

Inhibition by wild-type E1a. The significant structural differences

between the activating region of E1a and those of other proteins, suggested that E1a might interact with a unique cellular target. To investigate the nature of this target we performed squelching experiments similar to those that have been carried out with other activators^{11,14,21}.

GAL4-E1a can efficiently activate the GAL4-E1b TATA reporter plasmid, whereas wild-type E1a cannot⁴. Presumably, this is because wild-type E1a is not efficiently directed to this synthetic promoter. We could therefore use wild-type E1a to compete with GAL4 derivatives for the target of its activating region. High levels of E1a inhibited transcription directed by the GAL4-E1a activator (Fig. 3a). In contrast, activation by strong (GAL4-VP16 and GAL4-regII) or weak (GAL4-regI) acidic activators was either unaffected or not significantly inhibited by coexpression of E1a (Fig. 3a). These results suggest that the activating region of wild-type E1a protein competes for the target of the identical activating region carried by GAL4-E1a. They further suggest that E1a's target is not required by the acidic activators, regions I and II of GAL4 or VP16.

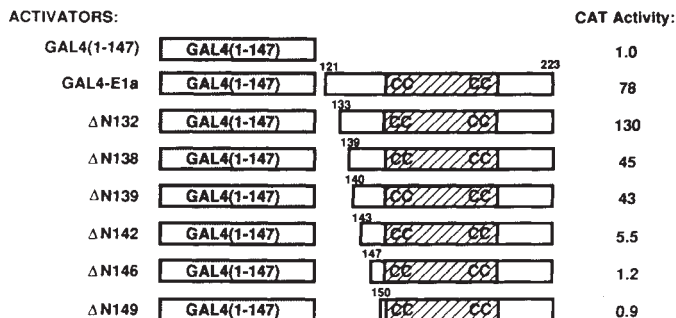
Immunoprecipitation and immunoblot experiments indicate that the various GAL4 derivatives (GAL4-E1a, GAL4-VP16, GAL4-regII and GAL4-regI), which contain the same 147 N-terminal amino acids, were expressed at comparable levels (data not shown; see refs 4, 15). Moreover, when the amount of transfected DNA encoding the activator was reduced nearly 1,000-fold, the same specificity of inhibition was observed (see Fig. 6). Thus, the specificity of inhibition cannot be accounted for by variations in the levels of the different activators.

A prediction of these results is that GAL4-E1a would not be able to activate transcription in cells that express high levels of wild-type E1a. Figure 3b shows that in 293 cells, GAL4-VP16 but not GAL4-E1a can activate transcription from the GAL4-E1b-TATA reporter.

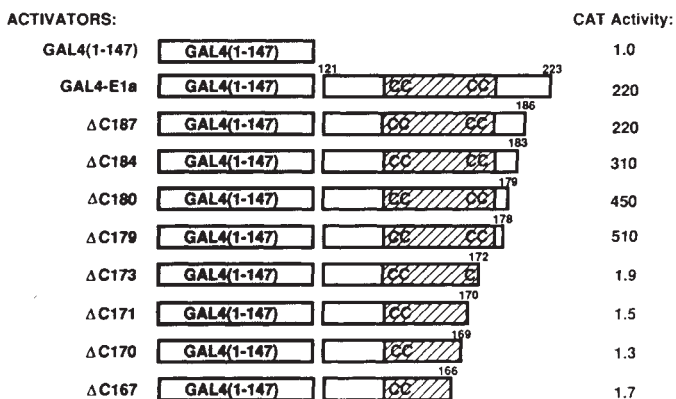
FIG. 1 The E1a transcriptional activating region lies between amino acids 140 and 178. A reporter CAT plasmid containing a core promoter (E1b TATA box) and two or five GAL4 binding sites was cotransfected with a plasmid encoding an activator protein. Relative CAT activity was measured and is indicated next to the diagrammed activators. The reporter plasmids are diagrammed below the sets of activators.

METHODS. Each GAL4 binding site is the 17-base pair (bp) oligonucleotide (MH100) previously described³³ and is inserted immediately upstream of each target promoter as indicated. The E1b TATA promoter contains an oligonucleotide comprising the E1b TATA box (5'-AGGGTATATAATG-3') inserted immediately upstream of the CAT gene in pSP72. GAL4 (1-147) is expressed from plasmid pSG147 or pSG424 (ref. 15). GAL4-E1a (pSGE1a) is E1a amino acids 121-223 fused in frame to the C-terminus of GAL4 (1-147), and was constructed by linking the *Clal*-*XbaI* fragment of an adenovirus 5(Ad5) E1a 13S cDNA plasmid³⁴ downstream of GAL4 (1-147). GAL4-E1aΔN132, GAL4-E1aΔN138, GAL4-E1aΔN139, GAL4-E1aΔN142 and GAL4-E1aΔN146 are progressive 5' *Bal*/31 deletions of the 13S cDNA *Clal*-*XbaI* fragment, fused in frame to GAL4 (1-147). The GAL4-E1aΔC deletions are progressive 3' *Bal*/31 deletions of the 13S cDNA *Clal*-*XbaI* fragment, fused in frame to GAL4 (1-147). Unless otherwise stated, 2 μg per plate each of the reporter and activator plasmids were transfected by the DEAE dextran technique³⁵ into chinese hamster ovary (CHO-DUKX) cells grown on plates with a diameter of 10 cm. Cells were maintained below 50% confluency in α-MEM plus nucleotides and were split 1:8 24 h before transfection. Unless otherwise stated, 48 h post-dimethylsulphoxide shock cells were collected and assayed for CAT activity as described³⁶. After autoradiography of the separated acetylated chloramphenicol forms, spots were excised and quantitated by liquid scintillation counting. All assays presented have been repeated at least three times with comparable results.

AMINO-TERMINAL DELETIONS:



CARBOXYL-TERMINAL DELETIONS:



Sequences required for inhibition. If the inhibition is due to squelching, then the sequences required for inhibition should be identical to those required for activation. To delineate the E1a sequences required for inhibition, we used as inhibitors the GAL4-E1a derivatives described above and assayed expression levels (Fig. 2c). We used a reporter chloramphenicol acetyl transferase, (CAT) plasmid that contained six LexA sites upstream of the E1b-TATA box and was therefore activated by LexA-E1a but not by GAL4-E1a. The LexA-E1a activator contains the DNA-binding domain (amino acids 1-202) of the *E. coli* LexA protein fused to E1a (amino acids 121-223) (Fig. 3c).

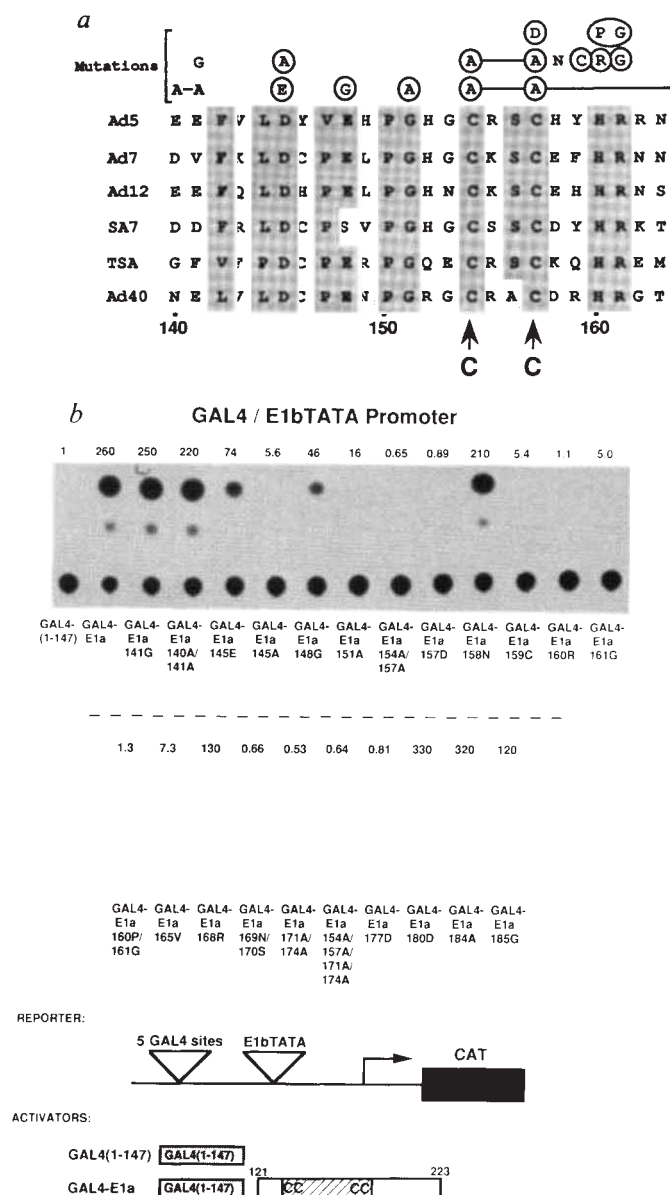


FIG. 2 Effects of amino-acid substitutions within Ad5 region 3. **a**, Region 3 amino-acid sequences (single-letter code) of six different adenoviruses^{37,38}. The shaded regions represent those amino acids that are either strictly conserved in at least five of the six adenoviruses, or that have undergone conservative changes. The numbers below the sequences refer to the amino-acid position of Ad5. The bold Cs beneath the sequences indicate the positions of the four cysteines that chelate Zn^{2+} . The letters above the adenovirus sequences represent single, double and quadruple amino acid changes we have constructed in Ad5 E1a region 3. Letters located above the mutated amino acid represent the residues that have been substituted. Lines connecting letters indicate that multiple amino-acid substitutions have been made within the same molecule; circles that include more than one letter indicate that all enclosed substitutions were made in the same

Mutations within the E1a activating region, including the metal-binding site, reduced the ability of GAL4-E1a to inhibit LexA-E1a function. In contrast, mutations outside the E1a activating region did not affect inhibition by GAL4-E1a. Significantly, the degree to which each mutated activating region inhibited activation correlated with the strength of the mutated activating region. The cysteine mutations, for example, which completely eliminated activation (Fig. 2b), also completely eliminated inhibition (Fig. 3c). The E1a mutation 145A, which caused an intermediate level of activation (Fig. 2b), also caused an intermediate level of inhibition (Fig. 3c). We have tested all mutations in Fig. 3a that decrease activation and the rank order

molecule. Letters not connected by lines or circled together represent single-residue substitutions. Those substitutions that had a greater than threefold effect on the ability of GAL4-E1a to activate transcription from a promoter containing GAL4 sites are circled. **b**, Transcriptional activity of mutant GAL4-E1a proteins. A reporter CAT plasmid containing a core promoter (E1b TATA box) and five GAL4 binding sites was cotransfected with a plasmid encoding an activator protein. Relative CAT activity was measured and is indicated above each lane of the autoradiogram. **c**, Expression levels of mutant GAL4-E1a proteins. ^{35}S -labelled GAL4-E1a derivatives from transfected COS cells were immunoprecipitated with an anti-GAL4 (1-147) antibody and fractionated on a polyacrylamide gel. **METHODS.** **b**, GAL4-E1a180D contains a substitution at E1a amino acid 180 (G $\rightarrow$ D) and was constructed by replacing the *Clal*-*XbaI* fragment of GAL4-E1a with the *Clal*-*XbaI* fragment of the E1a activation mutant pH3G-13S-1098. All other mutations within region 3 were generated by site directed mutagenesis with mismatching oligonucleotides³⁹ or by random chemical mutagenesis⁴⁰. **c**, COS cells were used to quantitate protein levels since higher levels of proteins are produced, facilitating detection. COS cells were transfected as described in the legend to Fig. 1. At 72 h post-transfection, cells were incubated with $200 \mu Ci ml^{-1}$ of [^{35}S]methionine for 3 h. Labelled GAL4-E1a derivatives were immunoprecipitated⁴ with an α -GAL4 (1-147) antibody (a gift of I. Sadowski) and fractionated on a 10% polyacrylamide gel. Unlabelled whole cell extracts were also prepared from COS cells cotransfected with plasmids encoding each activator and the GAL4-E1b-TATA-CAT reporter. These were assayed for CAT activity and results were identical to those obtained in CHO cells (data not shown).

in which they affected inhibition was the same as the rank order in which they affected activation (Fig. 2b, 3c, and data not shown). We conclude that the same E1a sequences are required for activation and inhibition. (This inhibitory effect is unrelated to the previously described E1a-mediated repression of enhancer-dependent transcription, which does not require conserved region 3 sequences²²⁻²⁴.)

Figure 4 presents a control experiment which shows that coexpression of wild-type E1a with an E1a fusion protein (in this case LexA-E1a) did not alter the amount of fusion protein capable of binding to DNA. This assay made use of a reporter with a LexA site located between the TATA box of the SV40

promoter and a *CAT* gene. Proteins that bind to this LexA site inhibit transcription³⁵. Under the identical conditions that resulted in a large inhibition by the wild-type E1a of LexA-E1a activation (Fig. 4, left panel), there was no change in the level of DNA binding by LexA-E1a (Fig. 4, right panel). At the level of LexA-E1a that was used (0.5 µg DNA), this binding assay was sensitive to changes in protein levels (Fig. 5, bottom panel of graph). Neither LexA-E1a nor wild-type E1a affected transcription from an SV40-*CAT* reporter lacking LexA sites, and wild-type E1a did not affect transcription from the SV40-LexA-*CAT* reporter (data not shown).

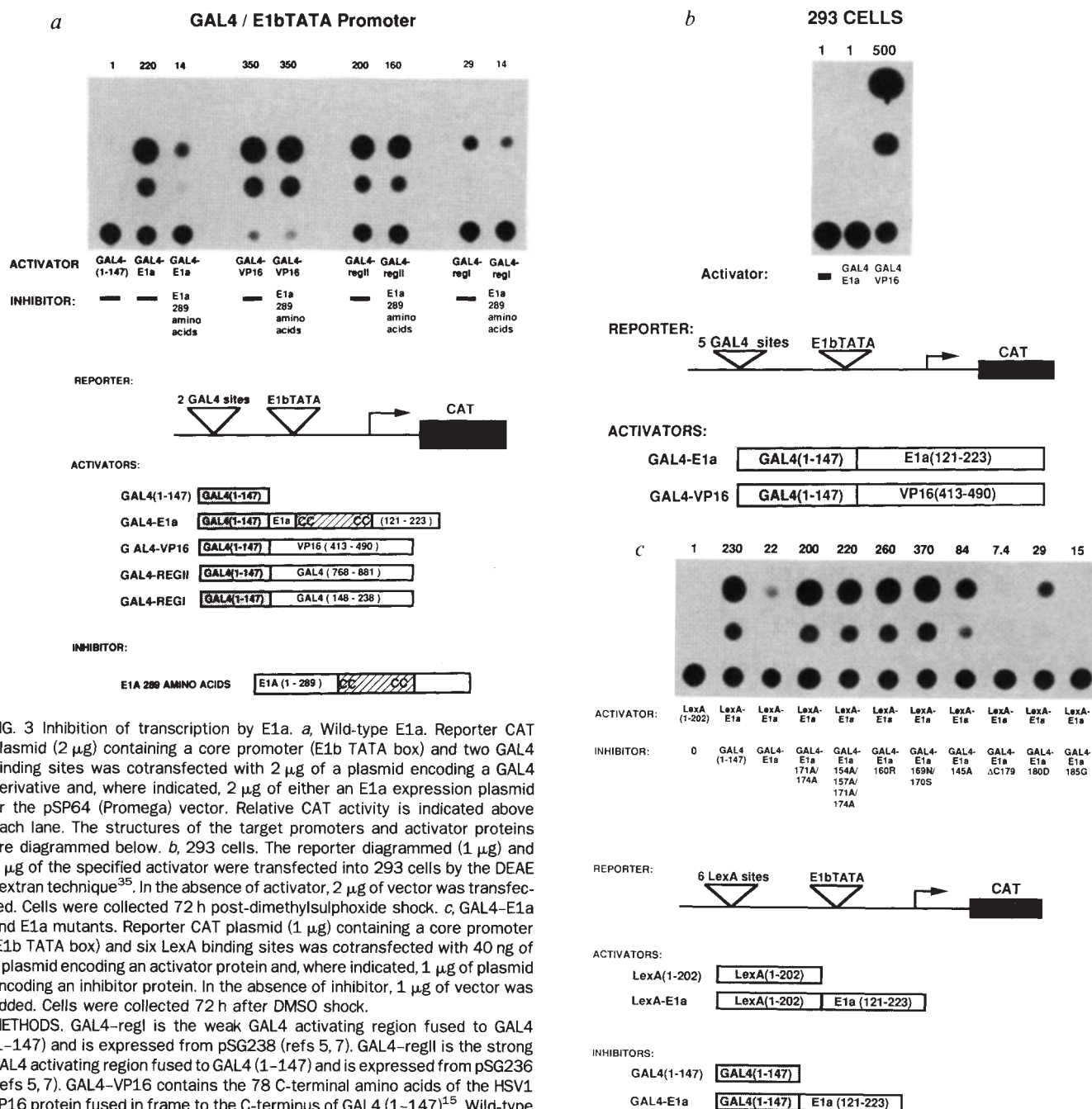


FIG. 3 Inhibition of transcription by E1a. *a*, Wild-type E1a. Reporter *CAT* plasmid (2 µg) containing a core promoter (E1b TATA box) and two GAL4 binding sites was cotransfected with 2 µg of a plasmid encoding a GAL4 derivative and, where indicated, 2 µg of either an E1a expression plasmid or the pSP64 (Promega) vector. Relative *CAT* activity is indicated above each lane. The structures of the target promoters and activator proteins are diagrammed below. *b*, 293 cells. The reporter diagrammed (1 µg) and 2 µg of the specified activator were transfected into 293 cells by the DEAE dextran technique³⁵. In the absence of activator, 2 µg of vector was transfected. Cells were collected 72 h post-dimethylsulphoxide shock. *c*, GAL4-E1a and E1a mutants. Reporter *CAT* plasmid (1 µg) containing a core promoter (E1b TATA box) and six LexA binding sites was cotransfected with 40 ng of a plasmid encoding an activator protein and, where indicated, 1 µg of plasmid encoding an inhibitor protein. In the absence of inhibitor, 1 µg of vector was added. Cells were collected 72 h after DMSO shock.

METHODS. GAL4-regI is the weak GAL4 activating region fused to GAL4 (1-147) and is expressed from pSG238 (refs 5, 7). GAL4-regII is the strong GAL4 activating region fused to GAL4 (1-147) and is expressed from pSG236 (refs 5, 7). GAL4-VP16 contains the 78 C-terminal amino acids of the HSV1 VP16 protein fused in frame to the C-terminus of GAL4 (1-147)¹⁵. Wild-type E1a is the 289-amino-acid E1a protein expressed from a plasmid (pSP613S) that contains the E1a region of adenovirus type 2 (minus the 13S intron) inserted into pSP64 (Promega). The LexA-E1bTATA reporter (pL6EC; a gift from P. Broad) has a triplicated insert of a 41-bp *Xho*I fragment, which contains two LexA sites derived from the *colE1* promoter⁴¹ in the *Xho*I site of the E1b TATA reporter. LexA (1-202) comprises LexA amino acids 1-202 and 12 amino acids coded for by a polylinker and is expressed from the

plasmid pBXL1 (gift from P. Broad). This plasmid is analogous to pSG424 (ref. 42), but contains LexA (1-202) in place of GAL4 (1-147). LexA-E1a is E1a amino acids 121-223 fused in frame to the C-terminus of LexA (1-202) and was constructed by inserting the *Eco*RI-*Xba*I fragment of pSGE1a (GAL4-E1a) downstream of LexA (1-202), between the *Eco*RI and *Xba*I sites of pBXL1.

Self-squelching of E1a derivatives. We next determined the effect of overexpressing a single E1a derivative. Figure 5 shows that increasing the amount of transfected DNA expressing either GAL4-E1a or LexA-E1a led at first to increased activation and subsequently to inhibition. The bottom panel of the graph confirms that the level of LexA-E1a protein correlated with the amount of transfected DNA. The intracellular level of LexA-E1a protein increased steadily over the course of the titration as assayed by its ability to bind *in vivo* to a LexA site located between the SV40 promoter and a CAT reporter gene²⁵. Proteins that bind to this LexA site inhibit CAT expression. Expression from an SV40-CAT reporter that lacks LexA sites was unaffected by cotransfection of the plasmid expressing LexA-E1a (data not shown).

These results suggest that, as the intracellular concentration of the E1a derivative is raised, activation of transcription increases until all DNA sites or all activation targets are saturated. After that, overexpression of the E1a derivative reduces the level of activation as the unbound E1a derivative begins to sequester the activating target. Self-squelching by LexA-E1a and GAL4-E1a show that E1a squelching is not a consequence of the coexpression of two different E1a derivatives (for example, by formation of inactive heterodimers).

Cellular target of the activating region of VP16

Expression of the E1a activating region can inhibit transcription directed by GAL4-E1a or LexA-E1a but not by the acidic activating regions of GAL4-VP16, GAL4-regI or GAL4-regII (Fig. 3). This indicates that the target of the E1a activating region is not required for transcription directed by acidic activators. To address whether acidic activators use targets that are required for transcription directed by E1a, we asked whether VP16 can squelch E1a.

Figure 6 shows that, as expected, VP16 can compete for its own activation target: LexA-VP16 inhibited transcription activation by GAL4-VP16. The target of VP16's activating region is also required for activation by E1a: LexA-VP16 inhibited activation by GAL4-E1a. As with the full-length E1a protein, an E1a fusion protein competed for its own activation target

(LexA-E1a inhibited GAL4-E1a), but did not inhibit transcription directed by the VP16 activating region. We are not merely observing an ability of the inhibitor to squelch any activator of equal or weaker strength than itself, as neither E1a nor LexA-E1a can squelch the relatively weak GAL4-regI, or GAL4-regII (Fig. 3a and data not shown). Thus, we conclude that E1a's activation target is not required by VP16, whereas VP16's activation target is required by E1a.

Discussion

Activating region of E1a. The activating region of E1a has relatively discrete boundaries and is inactivated by a number of single amino-acid substitutions. These essential amino acids are of several classes. Taken together, our mutagenesis experiments suggest that the E1a activating region is highly structured and that this structure is important for activity. It is likely that the metal-binding site, which is essential for function, has an important structural role. Metal-binding sites can allow proteins to form highly structured and essentially independent domains from a limited number of amino acids²⁰.

Many sequence-specific DNA-binding proteins contain metal-binding sites, termed Zn fingers^{19,20,26}. It is not clear whether E1a's metal-binding site is involved in DNA binding. Although E1a is not a sequence-specific DNA-binding protein,

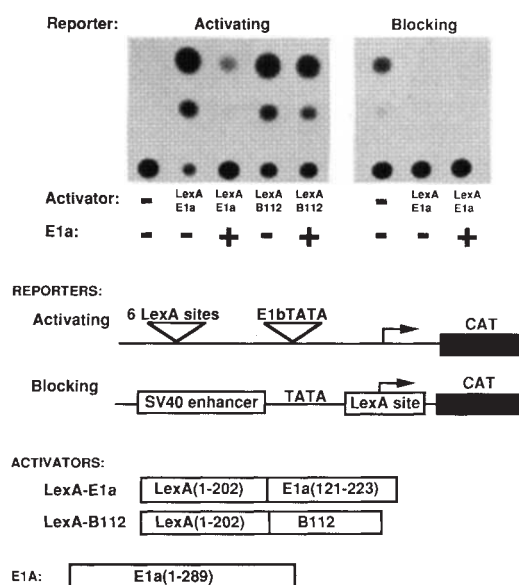


FIG. 4 *In vivo* DNA-binding assay. The diagrammed reporter (1 μ g), 0.5 μ g of the specified activator or vector DNA, and 1 μ g of E1a expression plasmid or vector DNA were transfected into CHO cells. Cells were collected 56 h post-DMSO shock. The two panels represent transfections done in parallel with identical procedures, only the reporter DNAs differ. LexA-B112 (a gift from P. Broad) is LexA (1-202) fused at its C terminus to B112, which is an acidic amphipathic amino-acid sequence derived from random *E. coli* DNA that functions as an activator of transcription¹⁰.

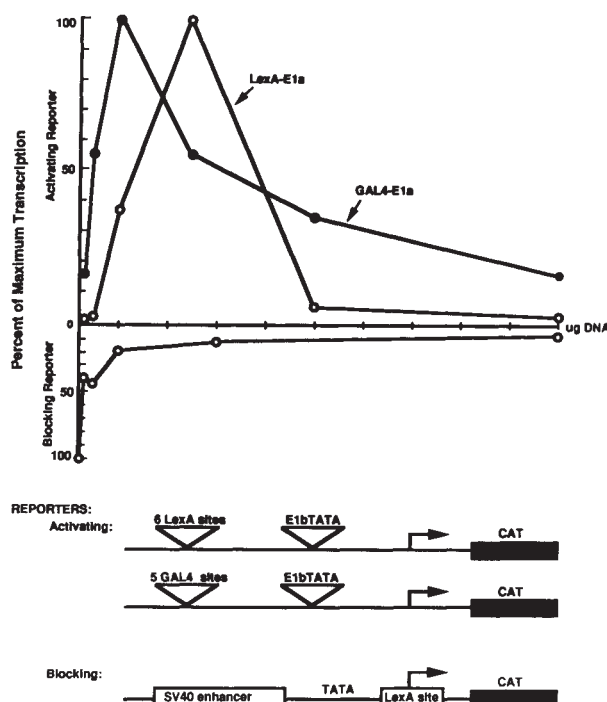


FIG. 5 High levels of either GAL4-E1a or LexA-E1a inhibits transcription activation. Activating reporter CAT plasmids (1 μ g) containing a core promoter (E1b TATA box) and either six LexA sites or five GAL4 sites were cotransfected with varying amounts of plasmids encoding the appropriate E1a fusion protein, LexA-E1a (○) or GAL4-E1a (●). The total amount of DNA transfected was held constant by including an appropriate amount of vector DNA. Each mark on the x-axis represents 1 μ g of activator DNA; a minimum of 100 ng and a maximum of 10 μ g of activator DNA were transfected per 10 cm plate of cells. Cells were collected 72 h post-dimethylsulphoxide shock. Relative CAT activity was measured and is graphed as a percentage of the maximum level of CAT activity for each DNA titration. The data plotted below the x-axis are CAT expression levels from a cotransfection of the blocking reporter diagrammed, also called VV(X)CO²⁵, and varying amounts of DNA encoding LexA-E1a. Relative CAT expression levels are reported as a percentage of the level of CAT expression observed in the absence of LexA-E1a. The lower plot is an indication of the intracellular concentration of the LexA-E1a protein. Structures of the target promoters are diagrammed below.

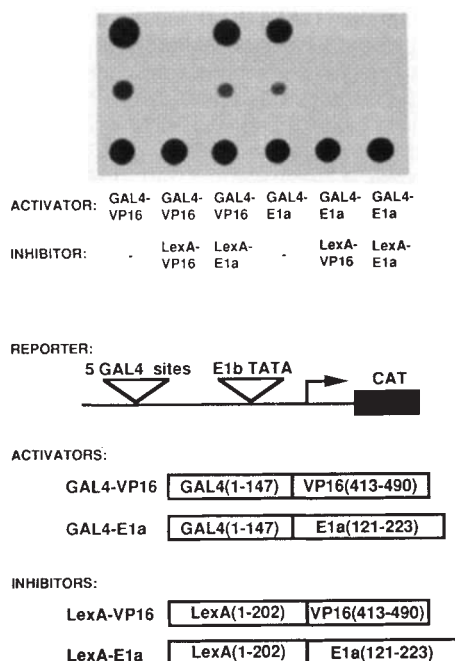


FIG. 6 Inhibition by VP16 and E1a transcriptional activating regions. A reporter CAT plasmid (1 μ g) containing a core promoter (E1b TATA box) and five GAL4 sites was cotransfected with plasmids encoding an activator and, where indicated, an inhibitor protein. 3 ng of the GAL4-VP16 activator and 10 ng of the GAL4-E1a activator were used. The inhibitors were both used at a level of 3 μ g.

METHODS. LexA-VP16 (gift from P. Broad) and contains the 78 C-terminal amino acids of HSV1 VP16 protein fused in frame to the C-terminus of LexA (1–202) in the expression vector pBXL1. Cells were collected 72 h post-DMSO shock. CAT assays of the GAL4-E1a activators (lanes 4–6) were performed with 25-fold more cell extract than those of the GAL4-VP16 activator (lanes 1–3).

it may bind to DNA nonspecifically²⁷. The E1a region responsible for DNA binding and its relevance to transcription remains to be determined. Berg²⁰ has pointed out that E1a's metal-binding site bears structural similarities to the metal-binding site of aspartate transcarbamylase, which is not a DNA-binding protein. The metal-binding site of aspartate transcarbamylase is at a surface involved in protein-protein interaction²⁸. We favour the idea that the principal role of E1a's metal-binding site is to form a surface that interacts with other proteins.

Adaptors. We suggest that the inhibition observed at high concentrations of the E1a activating region is caused by sequestration of its cellular target. Our interpretation is like those reached in studies of other activating regions^{11,14–16}. We recognize that neither our nor previous results prove the existence of a limiting cellular target, or that competition for it caused the inhibition. We cannot, however, think of an alternative interpretation that adequately explains these results. Moreover, our demonstration that there is specificity to squelching significantly strengthens this interpretation as it rules out the possibility that the inhibition is caused by cytotoxicity or a nonspecific inhibitory effect on transcription, for example.

We refer to E1a's target, inferred from these experiments, as

an 'adaptor' because it is not required for transcription directed by acidic activators. E1a's target is therefore an auxiliary, rather than an essential, transcription component. In contrast, the target of acidic activating regions is required for transcription directed both by VP16 and by E1a. This could be explained if acidic activating regions directly interact with an essential transcription component. In fact, DNase I footprinting studies provide evidence for an interaction between an acidic activating region and the TATA-box binding factor, TFIID²⁹.

We imagine that E1a's adaptor binds to E1a and also interacts directly with the basic transcription machinery. For example, the adaptor may contain, in addition to an E1a binding surface, an acidic activating region. A precedent for such an adaptor is the viral VP16 protein, which contains a potent acidic activating region^{15,16} and a second region that interacts with the cellular transcription factor Oct-1^{30,31}. By itself, Oct-1 cannot activate transcription from a typical promoter containing a TATA box. The same promoter can, however, be activated by Oct-1 in the presence of VP16³². It is conceivable that other nonacidic activating regions, such as those rich in glutamines or prolines, may also act through specific adaptors. Such adaptors would explain how diverse protein motifs can all activate transcription through the same basic transcription components. □

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Opaque spiral galaxies

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SPIRAL-galaxy disks have long been thought to contain only a modest amount of light absorbing dust, meaning that they are transparent to their own starlight, and that observers see light from their far sides. This view was based on studies of the variation of the average surface brightness as a function of the galactic inclination along the line of sight in small samples of galaxies^{1,2}. We³ have digitized the photographic images of a carefully selected sample of about 16,000 galaxies and determined an extensive set of photometric parameters. These data allow a re-analysis of the effective transparency, using the measured surface brightness profiles for much larger, more strictly defined samples. Several tests provide evidence that the major parts of many spiral disks are opaque, and that in many cases perhaps only the outer layer of stars is observable. This invalidates most determinations of mass-to-light ratios. Various photometric properties point to an obscuring component with a larger exponential scale length than that of the stars, possibly composed of cool compact opaque clouds. This result diminishes the evidence for dark haloes around spirals, as inferred from rotation curves.

Classically, the extinction inside the disks of spiral galaxies is studied by selecting a group with supposedly similar intrinsic properties and analysing how various parameters change with the inclination relative to the line of sight, the inclination angle being derived from the observed axial ratio a/b . For a transparent disk the apparent total blue magnitude B is constant, whatever the inclination, but for an optically thick disk, B varies approximately as $B_{\text{obs}} = B_{\text{face}} + 2.5 D \log(a/b)$, where $D \approx 1$ for a fully opaque disk. The variation of the local surface brightness μ^B as a function of inclination is reversed. For the optically thick case, μ^B is constant with inclination because the observer will always see about one mean free path into the system. In the transparent case, the longer line of sight through inclined systems will brighten the apparent μ^B approximately as

$$\mu_{\text{obs}}^B = \mu_{\text{face}}^B - 2.5C \log(a/b) \quad (1)$$

where $C = 1$ if there is no extinction at all.

Disney *et al.*⁴ recently reviewed the weak evidence for transparency of spiral galaxies and pointed out that various standard models^{1,2,5,6} correspond to values of C in the range 0.46–0.9 (semi-transparent to transparent). The models are based on Holmberg's original studies¹ of the relationship between the 'projected surface brightness' μ_{proj}^B and axial ratio for a sample of 119 spiral galaxies. He measured the total magnitude within the $\mu^B = 26.5$ isophote together with the major and minor diameters a and b , and from these derived $\mu_{\text{proj}}^B = B + 2.5 \log a^2$. Anticipating semi-transparent systems, he successfully fitted an 'absorbing screen' model to μ_{proj}^B as a function of a/b . If disks are fully opaque, however, a is not expected to vary with inclination. This implies that the variation of μ_{proj}^B with a/b can in fact represent the expected variation of B in the opaque situation. For axial ratios up to $a/b = 5$, Holmberg's original data points can be fitted quite well with an exclusive (opaque) $D = 1$ correction to B . With the thousands of data points from the ESO-LV survey (ref. 3), Holmberg's measurements of the regression of μ_{proj}^B with a/b is confirmed, but this classical test, which for several decades has been thought to indicate that the disks of spiral galaxies are transparent, can be interpreted equally well using the optically thick model. The ambiguity arose because of the use of the average surface brightness to study a/b -dependent terms, but now measures of the local surface brightness are available.

Here I report tests using the diameter-complete sample of 9,381 late-type galaxies extracted from the ESO-LV survey, that

is, a sample of galaxies for which full surface-brightness profiles have been measured. Three measures were used: the extrapolated central surface brightness $\mu_0^{B,\text{fit}}$ found by fitting an exponential to the two-dimensional light distribution, the μ_0^B within a $1''$ aperture converted from the measured surface brightness within an aperture of radius $5''$, $\mu_{5''}^B$, and the observed surface brightness at the half-total-light radius μ_e^B . Table 1 lists the means of these three independently derived values of the central surface brightness for different morphological types. For pure exponential disk systems without a bulge, $\mu_0^B = \mu_e^B - 1.822$. Columns 4, 6 and 7 demonstrate the consistency between the three different techniques and column 5 shows the very small standard deviation of μ_0^B . A comparison of μ_0^B with the other two measurements shows that the presence of bulges in early-type spirals (types 1 and 2) increases the actual central flux by only $\sim 0.3 m$ (m is relative magnitude); but results for these types could be affected by bulges. For the later types, no significant contribution from bulges is observed.

The key test, the analysis of the relationship between surface brightness and axial ratio, is presented in Fig. 1. This provides the strongest evidence that the disks of spiral galaxies are not transparent and, as the surface brightness at the half-total-light radius (diameter D_e) hardly brightens with a/b , this must be so for large parts of the disks. For different morphological types, equation (1) has been fitted to both μ_0^B and μ_e^B as function of $\log(a/b)$. The resulting coefficients, μ at $a/b = 1$ and C , are listed in Table 1. The central areas of all spirals of types 1–6 behave as would be expected for opaque conditions; this result was also found using the uncorrected $\mu_{5''}^B$. This condition seems to relax at the half-total-light radius of Sc and later types and at the centres of Sd galaxies. Beyond the half-total-light radius, any further radiation variation of C can be estimated using the distance-independent ratio D_{26}/D_e where D_{26} is the diameter

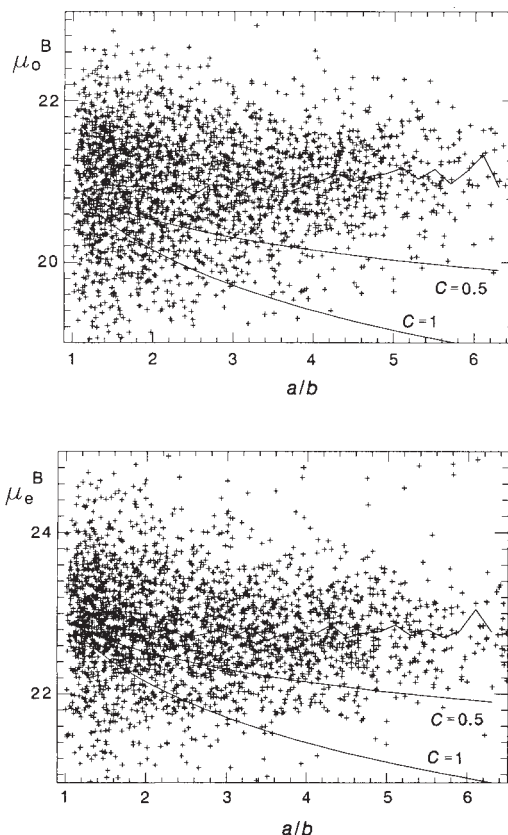


FIG. 1 The distribution of 2,639 type 2.5–5.0 (~Sb) galaxies in the surface brightness (left, at centre; right, at half-total-light radius) a/b diagram. The mean μ in a/b bins of 0.2 is indicated together with two curves representing the expected trend for transparent disks with $C=1$ and $C=0.5$.

TABLE 1 Comparison of different measures of central surface brightness of 9,381 late-type galaxies and deduced transparencies

Type	Total number	mean μ_0^B	s.d. μ_0^B	mean $\mu_e^B - 1.8$	mean $\mu_0^{B,fit}$	μ_0^B $a/b=1$	all C_{μ_0}	control C_{μ_0}	μ_e^B $a/b=1$	all C_{μ_e}	control C_{μ_e}	C_{1-D}	Number of controls
1 Sa	1,329	20.51	0.71	20.85	21.01	20.51	-0.01	-0.10	22.98	0.42	0.44	0.20	308
2 Sa-b	646	20.71	0.68	21.05	21.17	20.67	-0.06	-0.05	23.17	0.34	0.48	0.20	136
3 Sb	1,873	20.96	0.69	20.99	21.19	20.95	-0.02	-0.02	22.94	0.13	0.08	0.15	323
4 Sb-c	807	20.89	0.71	21.01	21.24	20.85	-0.08	-0.01	22.90	0.13	0.05	0.15	187
5	828	21.15	0.62	21.17	21.47	21.01	-0.18	-0.05	23.10	0.10	0.10	0.15	125
6 Sc	1,395	21.52	0.59	21.41	21.65	21.62	+0.10	+0.02	23.43	0.19	0.22	0.20	439
7	659	21.71	0.58	21.66	21.90	21.84	+0.14	+0.06	23.80	0.30	0.37	0.20	101
8 Sd	524	21.96	0.55	21.95	22.12	22.24	+0.32	+0.12	24.10	0.37	0.32	0.28	63
9	590	22.36	0.70	22.41	22.51	22.74*	+0.50*	+0.44	24.57*	0.57* [†]	0.27	0.28	81
10 Irr	730	22.27	1.03	22.59	22.34	22.85*	+0.76*	+0.41	24.62*	0.79* [†]	0.39	0.28	104
Dwarfs [‡] 3-7	100	21.34	0.66	21.24	21.49	21.39	+0.18	-	23.16	0.24			

 μ not corrected for galactic extinction.

* Using only exponential-profile systems.

[†] $C=0.3$ for whole sample.[‡] $M_B > -19$, Hubble constant $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$.

at $\mu^B = 26$ isophote. The a/b dependency of this ratio is related to the variation in transparency between the two diameters. The ratio is roughly constant for equal transparency and the ratio increases with a/b for all situations of radially increasing C . For types 2-6, no significant variation of D_{26}/D_e with a/b is found, which implies that the C_{μ_e} values listed in Table 1 must also apply to the outer regions of these galaxies.

The formal statistical errors of the listed C values are very small (≤ 0.03) and the implications of the low values obtained are so crucial for understanding the contents of spiral disks that a detailed evaluation of the tests is necessary. First, the tests were repeated using only those objects for which a free fit indicated that they possess a perfect exponential light profile. This reproduced the C values within the uncertainties and makes it very unlikely that the result is affected by the presence of bulges, at least for types 3-10. Second, the position of D_e could depend on a/b . In general, however, for both transparent and opaque systems the definition of D_e precludes such a dependency. As a check, C values were determined using $\mu_0^{B,fit}$, which resulted from a fit to the two-dimensional light distribution between $r = 5''$ and the 25th isophote. These values are insensitive to the actual location of D_e and reproduce those obtained from μ_e^B , within 0.03. Because for most systems D_e is located in the middle of the area used for the fit, this also confirms the small radial variation of C .

Finally, selection effects inherent in a diameter-selected sample³ may have influenced the result. At fainter magnitudes the survey has a bias against bright μ_0 and low- a/b systems (Fig. 2). By reducing the total sample to 1,867 objects, with $\mu_B < 21$ for types 1-5 and $\mu_B < 22$ for types 6-10, these selection effects are avoided. This 'control' sample has a distribution of observed a/b ratios very close to that expected from a randomly acquired sample of intrinsically round disks. Moreover, it is most complete with respect to its selection criteria. As seen in Table 1, the control samples reproduce the C values from the total sample very well, also demonstrating that axial-ratio selection effects are not affecting the result.

Another way of visualizing the result, using the total sample, is by counting the number of objects that are positioned on the right side of the curve $\mu_{lim} = 26$ in Fig. 2. An intrinsically round and face-on exponential disk galaxy will be a part of the sample if it lies to the left of this 'inclusion boundary'^{3,7,8}. All 4,873 galaxies (52%) on the right side of this curve, if they were intrinsically round, passed that boundary because of inclination effects. As their a/b is known the surface brightness and/or the magnitudes of these galaxies can be adjusted to their face-on values. When a 'transparent' correction ($C=1$) is applied to μ_0^B , 36% remain on the right side, for a 'semi-transparent' correction ($C=0.5$, $D=0.5$) the fraction is 23%, whereas for the 'fully opaque' correction on B_T ($D=1$), it is only 9%. As the curve describes the prime selection effect of a diameter-selected sample, this demonstrates that the data fully match the selection effect for opaque spirals, whereas transparent systems would lead to a serious inconsistency.

The low C values for large radii also imply a rather modest

dependency of isophotal diameters on a/b (contrary to ref. 5). This allows an independent check on the result, by analysing for which magnitude correction the scatter in the "absolute total magnitude intrinsic diameter" reduction is a minimum. For a subsample of 1,582 spirals a minimum scatter is found for $D=0.85$. For simple models, $C=1-D$, and estimates of C_{1-D} calculated from D are listed in Table 1. Magnitudes that were not corrected for inclination ($D=0$, transparent) resulted in a r.m.s. scatter of 0.64, whereas for an opaque ($D=1$) correction the scatter was reduced to 0.47. This reduction is highly significant (8.5σ) and confirms that most parts of the disks are opaque.

The tabulated C values can be used as an overall diagnostic on the effective transparencies at different locations for different types and can be applied directly to estimate face-on magnitudes for inclined systems. If $C \leq 0.2$ for large parts of the disks, as found for Sb and Sc galaxies, very strong obscuration is implied, requiring a dramatic revision of the interpretation of the surface-brightness profiles. For the centres, detailed modelling indicates that negative C values can occur, due to extinction of the light originating from opaque layers by surrounding semi-transparent layers. The amazingly small variation of μ_0^B (refs 9, 10) is also confirmed (contrary to refs 11, 12) albeit with the refinement that the average μ_0^B becomes larger with increasing type, which for later types (7-10) is also correlated with larger M . For opaque

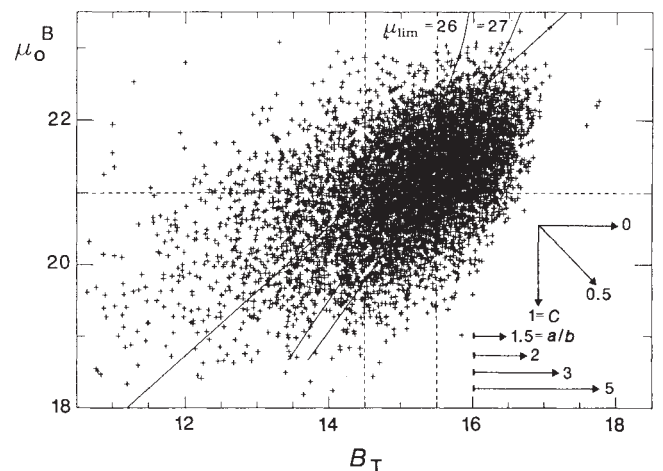


FIG. 2 A plot of central surface brightness against total magnitude for 8,061 spiral galaxies (type 0.5-8.5). Direct observational data, not corrected for inclination. The $\mu_{lim} = 26$ curve represents the theoretical inclusion boundary for circular face-on exponential disks and corresponds to the $1'$ -diameter selection criterion of the survey. The vectors show how intrinsically circular disks move in the diagram for various transparencies (direction) and inclinations (amplitude). The positions of 4,873 galaxies to the right side of the $\mu_{lim} = 26$ curve can only be understood if most of them represent inclined opaque disks. The straight line shows the observed regression for face-on circular disks. At fainter magnitudes the survey has a bias against bright μ_0 objects and low- a/b systems.

conditions, this small variation would result from a limited range of star-to-dust ratios for each morphological type, which on average should then also vary with type.

An observed value of $C = 0.2$ for large parts of Sb and Sc disks with a modest radial variation has even greater consequences. The observations cannot result from opaque layers with a small scale height compared to the stellar distribution, because the photometric properties of such disks would then be like those of transparent systems (μ_B decreasing with increasing a/b , contrary to the observations) and we would not even notice that we miss half of the starlight. A measurement of $C = 0.2$ at a particular radius could result from a two-layer situation in which 80% of the detected light originates from a fully opaque layer with optical depth $\tau \gg 1$ and the rest from a surrounding transparent layer. Calculations¹³ also demonstrate that the effect of multiple scattering on dust grains can produce a C value around 0.2 in $\tau \gg 1$ disks. Models incorporating a $\tau \gg 1$ layer would predict constant surface brightness profiles, however, given the observed modest radial variation of C . This also applies to the models of ref. 4 where the absorption is thought to be due to cirrus-like distributed dust. Areas of equal surface brightness can indeed be found along the arms of various spirals and a flat profile is also seen in several central areas. But many exponential light profiles are observed in $C = 0.2$ disks. This implies that the detected light and its corresponding C should originate mostly from a single layer of well mixed stars and obscurers, with $\tau \approx 1.3$ corresponding to ~ 0.6 m total face-on extinction, in which case about half of the system would be detected when face-on and one-sixth when at 80° inclination. The radial exponential intensity distribution should then be related to the radial volume density of the stars (ρ_*), the volume density ρ_0 , and cross-section σ_0 of the obscuring material by $I(r) \approx (\rho_*(r)/\rho_0(r)\sigma_0(r))(1 - e^{-\tau})$. For a modest variation of τ this implies either a radially decreasing star-to-obscurers density ratio, or increasing σ_0 , or both. In fact, the obscuring bodies must have the property $\tau = T\rho_0\sigma_0 = 1.3$, with T of the order of a hundred parsecs. This condition can be evaluated for all scales and the most reasonable match is with compact opaque clouds with, for instance, diameters of the order of 50 pc and $\rho_0 \approx 6 \times 10^{-6} \text{ pc}^{-3}$. These clouds should contain dust and stars that are in turn embedded in a sea of stars. As the distance to the galactic centres increases, the ratio of the volume density of the clouds to that of 'the sea of stars' should increase. The expected low temperatures for such clouds predict a substantial flux of submillimetre-wavelength radiation from Sb and Sc disks, which has indeed been observed¹⁴.

Whatever the true nature of the light-absorbing bodies, if they carry mass the implication is that the mass distribution in the disks will have a larger exponential scale length than that indicated by the observed light profile. In other words, the observed mass-to-luminosity ratio will increase with radius, despite the fact that the result implies an increase in true luminosities. This indicates a mass component that can produce flat rotation curves without the introduction of haloes of dark matter¹⁵. Successful fits of the rotation curve, with only disk material and obeying the extinction properties as presented here, have been made¹⁶. This result seriously weakens the evidence for dark haloes in Sb and Sc galaxies. Neither does it require alternative theories of gravity¹⁷ to explain the flat rotation curves. This result does not effect the evidence for dark haloes in elliptical galaxies^{18,19} (rotation velocities of which are, however, very difficult to explain with modification to Newton's law) or the missing-mass problem in spiral-poor clusters of galaxies, like the Coma cluster. $\square$

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Electronic properties of junctions between silicon and organic conducting polymers

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DIODES formed from semiconductor/metal interfaces often display non-ideal electronic properties. For instance, silicon/metal (Schottky) diodes made from n-type silicon and a variety of contacting metals exhibit only small differences in their rectification properties, despite theoretical and practical expectations that changes in the metal should effect changes in device properties^{1,2}. Similarly, Schottky diodes formed on p-type silicon generally exhibit ohmic behaviour with poor rectification characteristics. This lack of electrical response to changes in the properties of the contacting metal phase is generally attributed to interfacial reactions that take place during the high-temperature thermal or electron-beam deposition of metals onto silicon³. Here we describe the fabrication of diodes using a low-temperature chemical procedure, in which contact to the semiconductor is made by a layer of the conducting organic polymer, polyacetylene. Unlike conventional metals, the electrical properties of polyacetylene can be manipulated through choice of the polymer dopant. The resultant organic/inorganic interfaces behave more ideally than contacts with conventional metals, in that changes in the electrical properties of the conducting polymer exert a large and predictable effect on the electrical properties of the resulting semiconductor/polymer diodes.

Junctions were formed by preparing a layer of polyacetylene, $(\text{CH})_x$, on Si ($3.15 \Omega \text{ cm}$ (100) n-Si, or $2.45 \Omega \text{ cm}$ (111) p-Si, etched three times in 48% aqueous HF for 20 s, ref. 4) through the ring-opening metathesis polymerization of cyclooctatetraene⁵. Neat cyclooctatetraene was polymerized under nitrogen using the soluble tungsten carbene catalyst $\text{W}(\text{CHC}(\text{CH}_3)_3)(\text{NR})(\text{OR}_F)_2$, where R is 2,6- $\text{C}_6\text{H}_3(\text{CH}(\text{CH}_3)_2)_2$ and OR_F is $\text{OCCH}_3(\text{CF}_3)_2$ (ref. 6). The catalyst (8 mg) was added to the liquid monomer (100 μl) and the desired substrate coated with the resulting solution. After 10 min, an $\sim 300\text{-}\mu\text{m}$ -thick film of polymer formed on the Si surface. The excess monomer was removed under vacuum and the polyacetylene made conductive⁷ either by exposure to iodine vapour followed by vacuum pumping, producing $(\text{CHI}_{0.17})_x$, or by exposure to a potassium benzophenone ketyl solution in 2-methyl tetrahydrofuran, producing $(\text{CHK}_{0.06})_x$. Conductivities of these doped

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films were determined by standard four-point-probe methods; for $(\text{CHI}_{0.17})_x$, $\sigma = 150 \Omega^{-1} \text{cm}^{-1}$, and for $(\text{CHK}_{0.06})_x$, $\sigma = 50 \Omega^{-1} \text{cm}^{-1}$. Electrical contact to the polyacetylene was made by immersing a gold wire in the cyclooctatetraene; the wire became encapsulated by the polymer during polymerization, and was held in place satisfactorily by the set polyacetylene film. Separate measurements determined that the doped polymers made low-resistance ($< 50 \Omega$) contacts to gold. The polyacetylene devices were always handled initially in a dry nitrogen atmosphere to avoid the decomposition reactions known to occur on exposure of doped and undoped polyacetylene to air⁸. When devices were to be handled in air, degradation was prevented by sealing the junctions with a coating of epoxy. Using these procedures, no degradation of electrical properties was noticed until at least two weeks after junction formation.

Previous work has shown that the rectification properties of n-Si and p-Si junctions with conventional metals are relatively insensitive to the contacting metal, even though such behaviour is not expected based on the ideal Schottky model of these junctions¹⁻³. Figure 1 shows schematic energy diagrams for several types of Si junctions. In an ideal system, the position of the equilibrium Fermi level (E_F) in the Si is directly related to the work function of the contacting metal. The Si-band edge energies can be determined from the known values of the electron affinity of Si (4.05 eV) and the Si band gap (1.12 eV)². As can be seen in Fig. 1b, c, a change in the work function of the contacting phase should change the equilibrium position of the Si Fermi level. But for Si contacts to conventional metals, the Fermi level is found to be pinned to a small energy region. Metals ranging from copper to platinum (with work functions from 4.0 to 5.5 eV) all form low-barrier ohmic contacts to p-Si and rectifying junctions (with barrier heights of 0.7–0.9 eV) to n-Si, whatever the actual value of the metal work function (see Fig. 1a). High-barrier-height contacts to p-Si and low-barrier-height contacts to n-Si, as depicted in Fig. 1c, cannot be formed from direct Schottky contacts to these metals. Typical data for Si/Au junctions obtained in our laboratory are in agreement with these non-ideal properties (Fig. 2). The insensitivity of current density as a function of voltage (J - V properties) for a large variation in metal work function is termed Fermi-level pinning, and has generally been ascribed to the tendency of silicon to form metal silicides with most metals of interest^{2,9-11}. The metal silicide layer then determines the J - V properties of the semiconductor/metal contacts, and precludes manipulation of the device properties by changing the metal.

Figure 2a displays the J - V properties of p-Si/ $(\text{CHI}_{0.17})_x$ and p-Si/ $(\text{CHK}_{0.06})_x$ contacts. A unique feature of a conducting polymer such as polyacetylene is that it can be doped with either oxidants or reductants, making it a material with a variable work function. The high-work-function¹² $(\text{CHI}_{0.17})_x$ overlayer yields a poorly rectifying contact to p-Si, as expected from the ideal band-equilibration model presented in Fig. 1b. Similarly, the low-work-function¹³ $(\text{CHK}_{0.06})_x$ overlayer yields excellent rectifying properties on p-Si (Fig. 1c). A quantitative analysis can be made using the diode equation $J = J_0[\exp(qV/AkT) - 1]$, where k is the Boltzmann constant, T is the temperature, J_0 is the exchange (saturation) current density, A is the diode quality factor, and q is the electronic charge^{2,3}. Better rectification corresponds to lower values of J_0 and ohmic contacts result from high J_0 values. The p-Si/ $(\text{CHK}_{0.06})_x$ contact yielded a J_0 value of $6 \times 10^{-7} \text{A cm}^{-2}$, which is at least three orders of magnitude lower than the J_0 of conventional p-Si/metal contacts (typically 10^{-3}A cm^{-2} ; see Fig. 2 and ref. 2). In contrast, the p-Si/ $(\text{CHI}_{0.17})_x$ contact exhibited a J_0 value of $3 \times 10^{-3} \text{A cm}^{-2}$, providing a low-barrier contact.

The model in Fig. 1 also predicts that complementary behaviour should be observed with n-type Si as the semiconductor, where low-work-function contacts should yield ohmic contacts and high-work-function materials should provide rectifying diodes. Figure 2b displays the data for n-Si/ $(\text{CHI}_{0.17})_x$ and n-Si/ $(\text{CHK}_{0.06})_x$ contacts. The J_0 values for three separate n-Si/ $(\text{CHI}_{0.17})_x$ samples were 2.9×10^{-8} , 2.7×10^{-8} and $2.4 \times 10^{-8} \text{A cm}^{-2}$, whereas the n-Si/ $(\text{CHK}_{0.06})_x$ system had a poorly rectifying, ohmic J - V response, with a total resistance of 200 Ω . The range of J_0 values obtainable on conventional n-Si/metal interfaces is limited to about three orders of magnitude, whereas the J_0 values reported here span six orders of magnitude for the n-Si/ $(\text{CH})_x$ system (iodine and potassium dopants) and are in qualitative accord with the predictions of Fig. 1.

The behaviour of these conducting polymer/inorganic semiconductor interfaces is significant because it shows that restrictions on Schottky barrier devices can be overcome by using conducting organic polymer contact layers. Further, the ability to manipulate the properties of these interfaces by changing the polymer dopant allows switchable devices to be made. For instance, we have switched a device from rectifying to ohmic and back to rectifying behaviour by alternate iodine/alkali metal/iodine treatment of the $(\text{CH})_x$ layer of an n-Si/ $(\text{CH})_x$ contact. A few previous studies of polyacetylene contacts to inorganic semiconductors have been reported using

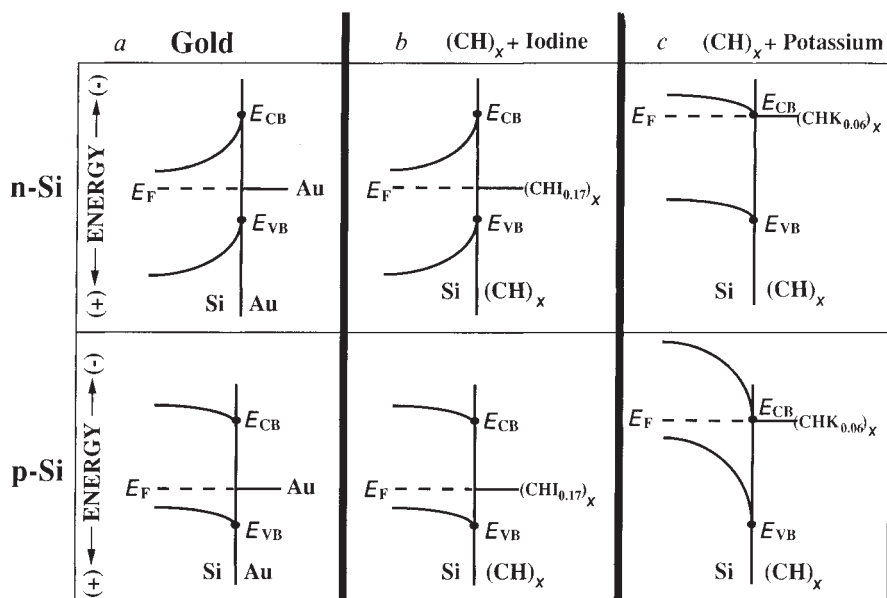


FIG. 1 Band-bending diagrams for n- and p-Si contacts to Au and $(\text{CH})_x$ (I_2 - and K-doped). The barrier height on n-Si is defined as the energy difference between the conduction-band edge, E_{CB} , of the Si and the equilibrium Fermi level, E_F , of the contact. The barrier height for a p-type semiconductor is equal to $E_{VB} - E_F$. The energies of the conduction band (4.05 eV) and the valence band (5.17 eV) are obtained from experimental values of the electron affinity and band gap of Si. See text for discussion.

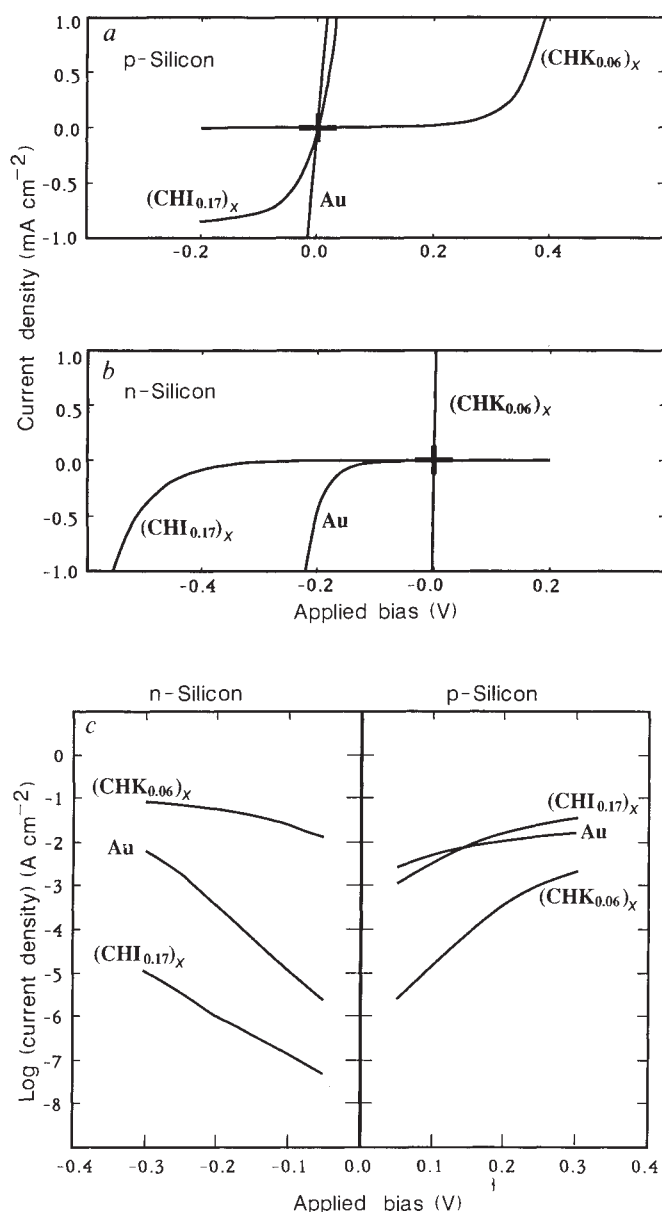


FIG. 2 Current density as a function of voltage for (a) p-type and (b) n-type Si/polyacetylene interfaces. The rectification characteristics change with the identity of the polymer dopant. The current-voltage curves for Si/Au diodes (typical of most Si/metal contacts) are added for comparison. The intercept (at zero applied bias) of the plot of log (current density) against forward bias (c) is a measure of the exchange (saturation) current density (J_0) for each interface. Curvature in the log plots at higher current densities arises from series-resistance effects in the diodes. The ratios of forward to reverse rectification (at ± 1 V) are: p-Si/(CHK_{0.06})_x = 6×10^3 , p-Si/(CHI_{0.17})_x = 2×10^2 , n-Si/(CHI_{0.17})_x = 5×10^4 . The diode quality factors are: n-Si/(CHI_{0.17})_x = 1.8, p-Si/(CHK_{0.06})_x = 1.2.

polyacetylene prepared by the Shirakawa route¹⁴⁻¹⁶, although the ability to manipulate the device properties by varying the polymer dopant was not described. The Shirakawa polyacetylene has been used in photoelectrochemical cells to inhibit photocorrosion and to mediate charge transport between the semiconductor and a solution redox species¹⁷, but the electrical properties of the isolated semiconductor/conducting polymer junction were not reported. New synthetic routes to polyacetylene have resulted in materials with superior processing and electronic characteristics. For instance, the recent fabrication

by Burroughes *et al.*¹⁸ of transistors and metal-insulator-semiconductor capacitors using polyacetylene as the active element relied on the Feast precursor method to generate homogeneous defect-free polyacetylene films^{19,20}. Our work uses the recently developed liquid-phase ring-opening metathesis method of polymerizing cyclooctatetraene, which is more controllable than the gas-phase polymerization of acetylene⁵. This allows for uniform coverage of material over the semiconductor surface and for facile control of the morphology of the layer of doped organic polymer. The ability to prepare junctions with well defined electrical properties is a key step towards the development of polymer-based electronic devices. □

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Coherent structures at the ocean surface in convectively unstable conditions

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THE turbulent boundary layer at the ocean surface has some dynamical similarities to the atmospheric boundary layer¹⁻⁴. The atmospheric turbulent boundary layer may exhibit not only random fluctuations but also spatially coherent, organized motion^{5,6}. Thorpe¹ conjectured that such organized motion should also be found in the upper ocean boundary layer in convectively unstable conditions. Here I report on observations made in the tropical Atlantic Ocean which confirm this view. Horizontal temperature profiles obtained at a depth of 2 m at night revealed ramp-like structures. Vertical velocity profiles in the upper few metres of the ocean was determined using a free-rising profiler, and exhibited abrupt changes corresponding to sudden changes in temperature. These features are known^{5,6} to be characteristic of spatially coherent, organized motions in turbulent boundary layers.

Laboratory experiments⁷ in a coordinate system moving with the average convection velocity of coherent structures show that the typical organized motion in the unstratified turbulent boundary layer resembles an inclined three-dimensional horseshoe vortex. In stratified conditions the vorticity features are accompanied by density ones. Measurements⁶ using a fixed probe in

TABLE 1 Observation of coherent structure in the upper ocean boundary layer

Run	Latitude	Date (1985)	Time (GMT)	U_0 (m s^{-1})	θ_0 (deg)	V_{10} (m s^{-1})	T_w (s)	H_w (m)	T_s (s)	H_s (m)	Q_R (W m^{-2})	Q_n (W m^{-2})	u_* (cm s^{-1})	T_* ($^{\circ}\text{C}$)	L_* (m)	μ_3
(a) Horizontal structure																
16 A, B	2° N	21 June	00:20–00:42	3.5		1*		ripples	5	1.0	0	–90	0.11	–0.049	–0.05	0.15, 0.43
16 C	2° N	21 June	00:42–00:52	3.5	0	1–3		<0.3	5	1.0	0					0.07
16 D, E, F	2° N	21 June	00:52–01:24	3.5	0	3.4	3	0.3	5	1.0	0	–140	0.38	–0.022	–1.3	–0.98, –1.00, –0.76
17 A, B, C	2–3° N	21 June	01:55–02:27	7.5	–55	3.0	3	0.3	5	1.0	0	–130	0.34	–0.023	–1.1	–0.41, –0.20, –0.18
13 A, B, C	2–1° N	20 June	14:18–14:50	7.5	37	3.0	3	0.3	5	1.0	840	–130	0.34	0.055	1.1	0.18, 0.18, 0.09
14 A, C, D	2–1° N	20 June	15:11–15:22	7.5	40	5.0	3	0.5	5	1.5	880	–200	0.56	0.028	2.3	0.12, 0.14, 0.13
			15:32–15:54													
14 E, F, G	2–1° N	20 June	16:01–16:33	7.5	45	6.3	3	0.5	5	1.5	800	–190	0.71	0.020	5.3	0.15, 0.09, 0.21
(b) Vertical profiles																
14–20	20° N	16 May	00:55–01:53	2.2		4.1	3	0.3	7	1.0	0	–180	0.46	–0.022	–1.9	
72–77	20° N	23 May	21:03–21:34	2.2		5.2	3	0.3	8	1.0	0	–200	0.59	–0.020	–3.6	

U_0 is the speed of probe relative to water, θ_0 the angle of ship's motion with respect to wind; V_{10} is the wind speed at a height of 10 m; T_w , T_s and H_w , H_s are the periods and the heights of wind waves and swell, respectively; Q_n is the solar insolation, Q_R the heat loss from the ocean surface owing to latent, sensible and long-wave radiative heat fluxes; $u_* = (\tau/\rho)^{1/2}$ is the friction velocity, where τ is the wind stress and ρ is the density of water; $T_* = Q_0/(u_* c_p \rho)$ is the friction temperature, where Q_0 is the heat flux in the upper layer of the ocean defined as $Q_0 = Q_n + (1-A)(1-f(z_0))Q_R$, $A=0.05$ is the albedo of the ocean surface, f is a function describing volume absorption of solar radiation in the upper layer of the ocean, $z_0=2$ m, $\kappa=0.4$ is von Karman's constant and c_p is the specific heat; $L_* = u_*^3/(\kappa \gamma g Q_0/c_p \rho)$ is the Monin–Obukhov length scale, where γ is the coefficient of thermal expansion of water and g is the acceleration due to gravity. Friction velocity was calculated as: $u_* = (\rho_a/\rho)^{1/2} C_{10}^{1/2} V_{10}$, where ρ_a is the density of air and C_{10} is the drag coefficient calculated in ref. 15, 1.04×10^{-3} . The latent and sensible heat fluxes were calculated from the air–ocean observations using standard bulk formulation¹⁶. Radiation fluxes were measured directly. Volume absorption of solar radiation was calculated as in ref. 17. Parameters of surface waves were obtained visually by a meteorologist.

* wind direction was unstable.

The convectively unstable atmospheric boundary layer show that the temperature has a characteristic ramp profile—a gradual rise followed by a relatively sharp decrease downstream—which leads to a skewed temperature derivative¹. The sharp change of temperature occurs when a frontal interface of the horseshoe vortex passes the temperature probe. The temperature ramps in the atmospheric boundary layer were suggested⁵ to be a signature of organized large-scale motion. In the surface ocean only the coherent structures of day-time stably stratified conditions have so far been observed^{1,8}.

An underwater probe⁹ was mounted on the bow of the RV *Akademic Kurchatov* at a depth of 2 m (Fig. 1). The probe measured the temperature and conductivity fluctuations ahead of the moving vessel. The uncertainty on the temperature measurement was $\sim \pm 0.001^{\circ}\text{C}$; the response time was 0.1 s. An acceleration sensor registered vertical motions of the probe caused by the pitching of the vessel. The acceleration signal was doubly integrated and processed by a digital high-pass cosine filter with a 0.02-Hz cut-off frequency (to avoid low-frequency trends) and therefore gives information about the depth variation of the probe with respect to a mean hydrostatic level of the ocean.

At the probe mount the radius of curvature of the vessel's hull is ~ 10 cm, with a frontal angle of 16° (Fig. 1). In front of a moving sphere the streamlines are not appreciably disturbed at

the distances greater than ~ 3 radii¹⁰. Owing to a 'knife-edge' hull, the *Akademic Kurchatov* produces practically no bow wave ahead of itself, as confirmed by photographs. No turbulent or wave perturbations from the vessel's body are therefore expected in the measurement zone.

A free-rising profiler⁴ (Fig. 1) measured vertical profiles of conductivity and the vertical component of velocity fluctuations in the frequency range 2–250 Hz. The vertical velocity of the profiler was 2.2 m s^{-1} . Temperature profiles were calculated from the conductivity profiles neglecting salinity variation^{4,11}. The uncertainties on the temperature and velocity fluctuations were $\sim 0.001^{\circ}\text{C}$ and 1 mm s^{-1} respectively. The profiler tends to follow the wave-induced orbital motion in the long surface waves⁴. This reduces the influence of surface waves on turbulence measurements in the upper boundary layer of the ocean; the vertical profiles may be found relative to a moving coordinate system following long waves (details are given in ref. 4).

Measurements with the underwater probe were made at 37° W between 1° N and 20° N in June 1985. At several points along this section the measurements were also made into the wind direction. Here I ignore data obtained when it was raining or in regions of oceanic fronts.

At night the temperature fluctuations in the upper ocean appreciably exceeded the noise level of the probe only at wind speeds $\leq 5\text{--}7 \text{ m s}^{-1}$. In particular the measurements made in the

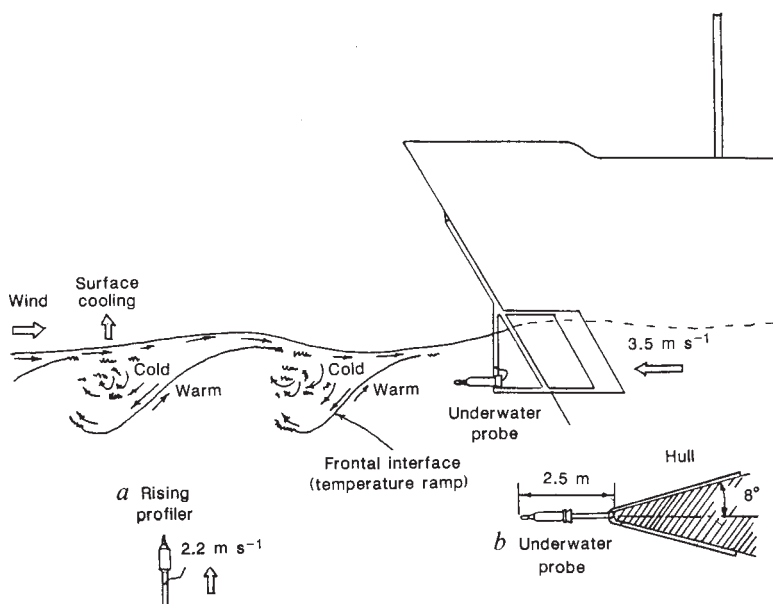


FIG. 1 Schematic representation showing spatially coherent organized motion in the upper ocean boundary layer and experimental techniques and devices used in measurements. a, Free-rising profiler, b, Top view at the probe mount (2 m below).

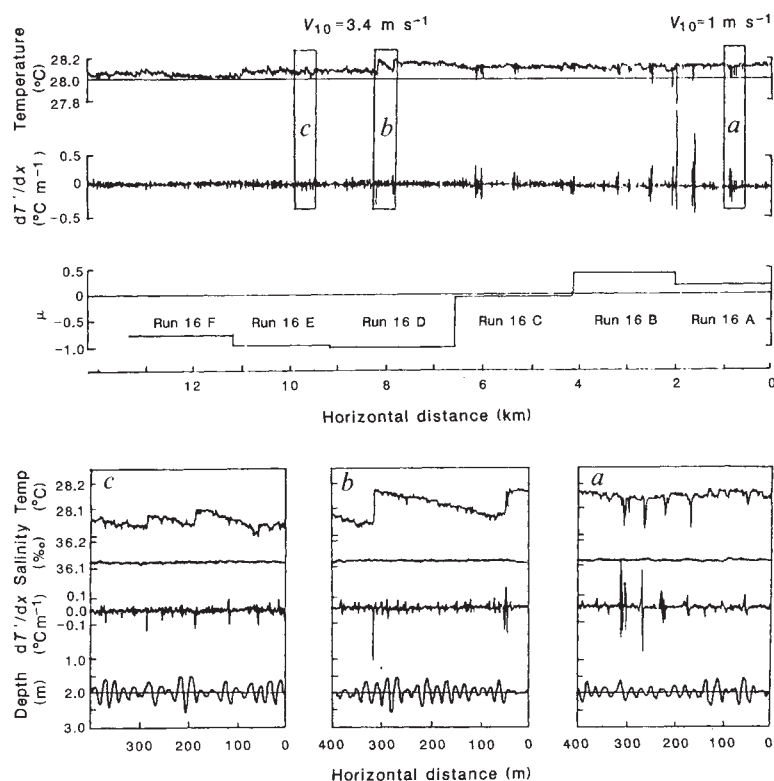


FIG. 2 Top, records of temperature (T), fluctuation-temperature derivative (dT'/dx) and skewness (μ_3) of the derivative obtained at 2 m depth in the early morning of 21 June (run 16 in Table 1). The graphs are plotted from right to left. Below, the marked regions (a , b and c) from above are shown in more detail along with the appropriate salinity records and depth variations of the probe relative to the mean hydrostatic level of the ocean, calculated from the accelerometer signal.

night of 20/21 June met this requirement (runs 16 and 17 in Table 1). Run 16 shown in Fig. 2 was made with the vessel heading into the wind at a speed of 3.5 m s^{-1} , after diurnal thermocline deepening. The real thickness of the seasonal mixed layer was $\sim 50 \text{ m}$. The initial part of the record (run 16 A, B) obtained in almost calm weather conditions was typical of buoyancy-driven convection ($z_0/L_* \approx -40$, where z_0 is the mean depth of the probe, L_* is the Monin-Oboukhov scale), isolated narrow regions of cooler water of $\sim 1\text{-m}$ scale (Fig. 2a) being ascribed to intersection of so called 'thermals' previously observed on the vertical temperature profiles in the convectively unstable upper boundary layer of the ocean^{11,12} and of a lake¹³. A ramp-like structure with negative skewness μ_3 of the tem-

perature derivative appeared in the temperature record (run 16, D-F in Fig. 2) after the wind speed increased. The value of $z_0/L_* \approx -1.3$ indicated that in this case the shear could have an important role.

Patterned on Fig. 4 of ref. 8, which shows processes of the vertical transport in a stably stratified shear boundary layer of the upper ocean, Fig. 1 reconstructs water circulation in unstably stratified conditions. As the ship moves upwind the ramps are observed as the probe passes from the 'warm water' to the 'cold water' side of the inclined sharp interfaces (Fig. 1). The ramps on the temperature record shown in Fig. 2 (run 16, D-F) are also consistent with this sketch.

The skewness of the temperature derivative for run 16 D-F falls in the range -0.7 to -1.0 . In the convectively unstable atmospheric boundary layer the skewness in the presence of the coherent structure is near the latter value¹⁴. As expected, the mean value of μ_3 measured in the night-time convectively unstable conditions depended on the direction of the ship's motion relative to the wind (compare run 16, D-F with run 17, A-C in Table 1). During the day the μ_3 became positive (see runs 13 and 14), the values being near those obtained previously^{1,8} in day-time stably stratified upper ocean in similar wind conditions and towing angles.

The vertical velocities measured by the free-rising profiler complement the ship-borne observations. This instrument was deployed 91 times in wind speeds of $2\text{--}7.5 \text{ m s}^{-1}$ in the vicinity of 20° N , 37° W . Thirteen measurements (7 on 16 May and 6 on 23 May) were made under weather conditions very similar to those during the runs on 20–21 June (see Table 1). Figure 3 shows a series of seven successive measurements of temperature and vertical velocity fluctuations made by the free-rising profiler in the early morning of 16 May. The time interval between successive measurements was $\sim 10 \text{ min}$ and the vessel wind drift during this period was several tens of metres. The investigated coherent structure had time and spatial scales of the same order, and therefore no determinable connection between any of the series profiles is expected.

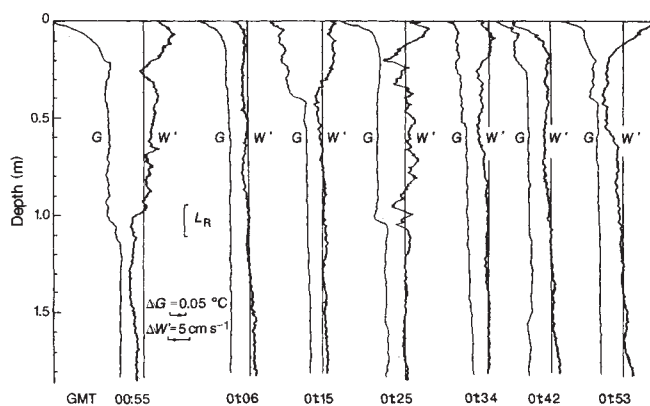


FIG. 3 Vertical profiles of temperature (G , calculated from conductivity profiles assuming constant salinity) and vertical component of velocity fluctuation (W') in the upper ocean in convectively unstable conditions. A positive $\Delta W'$ indicates a positive velocity change of the flow along the profiler. The vertical length scale (L_R) represents the relaxation time of velocity sensor, calculated using $L_R = W_0 / (2\pi f_1) = 17.5 \text{ cm}$, where $W_0 = 220 \text{ cm s}^{-1}$ is vertical velocity of profiler, $f_1 = 2 \text{ Hz}$ is lower frequency limit of the sensor.

The abrupt change of temperature and vertical velocity component at the depth of 1.08 m observed on the profiles obtained at 01:25 GMT (Fig. 3) can be ascribed to vertical intersection of a frontal interface (see the sketch on Fig. 1 showing eddies and frontal interfaces in the upper ocean boundary layer). The temperature change at this interface was $\Delta\theta \approx -0.05^\circ\text{C}$, the appropriate change of the vertical velocity being $\Delta W \approx 2.5\text{ cm s}^{-1}$. The sign of the vertical velocity change at 1.08 m depth indicated that the cold water was moving along the interface away from the ocean surface. This coincides with water circulation induced by the large-scale eddies in the upper ocean boundary layer (Fig. 1).

Similar profiles obtained at 00:55 GMT (Fig. 3), but having the opposite sign of the vertical velocity change at 0.95 m depth and the same sign of the temperature change as the profiles discussed above, may be interpreted as a result of intersection of the region where the cold water was moving along the frontal interface toward the ocean surface (see Fig. 1).

The profiles obtained at 01:06 GMT have no substantial changes either in temperature or in vertical component of velocity. These profiles can be interpreted as referring to a space between the large-scale eddies. The other four pairs of profiles

shown in Fig. 3 apparently are concerned with cases when the profiler passes through less pronounced regions of the organized vortex structure. Vertical profiles obtained on 23 May had similar temperature and velocity features. □

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Consumption of atmospheric methane by tundra soils

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EMISSION of methane from tundra soil contributes about 10% of the global atmospheric methane budget¹. Moreover, tundra soils contain 15% of global soil carbon², so the response of this large carbon reservoir to projected global warming^{3,4} could be important. Coupled biological models^{3–6} predict that a warmer climate will increase methane emission through increased rates of methanogenesis. Microbial oxidation of methane is, however, a possible control on emissions that has previously been overlooked. Here we report the results of field and laboratory experiments on methane consumption by tundra soils. For methane concentrations ranging from below to well above ambient, moist soils were found to consume methane rapidly; in non-waterlogged soils, equilibration with atmospheric methane was fast relative to microbial oxidation. We conclude that lowering of the water table in tundra as a result of a warmer, drier climate will decrease methane fluxes and could cause these areas to provide a negative feedback for atmospheric methane.

Methane is one of several radiatively active trace gases undergoing an atmospheric concentration increase of $1\% \text{ yr}^{-1}$ or more^{7,8}, and has the potential to cause climate change through atmospheric warming^{9–11}. Global methane budgets balance net

CH_4 sources to the atmosphere against the atmospheric photochemical sink, using ^{14}C and ^{13}C contents, and isotope fractionation factors as constraints^{1,12} on the source terms. Other methane sinks (consumption by soils and marine sediments) are incorporated in the net source terms and are not explicitly identified in these budgets. Some climate models incorporating greenhouse gas increases predict higher temperatures and drier summer conditions for high northern latitudes^{13–15}. Coupled biological models predict increased CH_4 emission as a result of increased rates of methanogenesis^{3–6}, but CH_4 oxidation was neglected in these models. Net consumption of atmospheric CH_4 has been reported in some soils of temperate forests^{16–18} and swamps¹⁹, tropical forests^{18,20–22}, savannah²³ and tundra^{24,25}. The areal extent of the soil CH_4 sink is poorly understood, but it is estimated to account for $<1\text{--}58 \text{ Tg yr}^{-1}$, or up to 11% of the global photochemical sink^{16,17,21}.

Our observations were made in a moist tundra meadow on Unalaska Island, Aleutian Islands, adjacent to Skan Bay (53°N , 167°W). Plant cover was a continuous mat of mosses invaded by lichens, cottongrasses (*Eriophorum* sp.), low-lying ferns and dwarf shrubs (*Vaccinium* sp.). The air temperature ranged between 5 and 8°C ; the soil temperature was 7°C and uniform in the upper 15 cm.

We collected syringe samples of gas from three static chambers at 0.25-h intervals and analysed them by gas chromatography²⁶ on board the RV *Alpha Helix*. The chambers were fitted with a capillary bleed to equalize pressure following sampling. Chamber measurements yield net fluxes—in the measurements reported here, the methane concentration decreased with time, indicating that consumption exceeded production in the moist

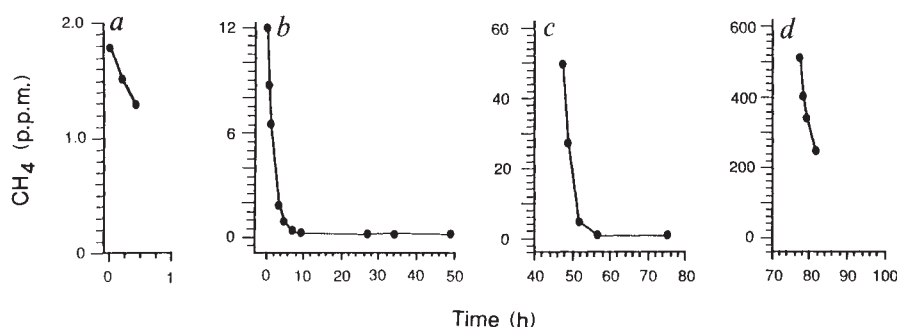
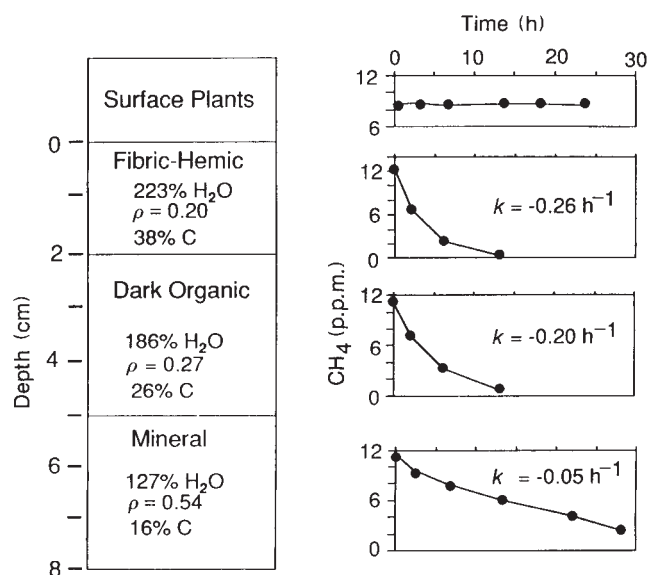


FIG. 1 Consumption of headspace methane in a field experiment using a static chamber. *a*, consumption of atmospheric CH_4 to sub-ambient levels on 8 October 1987. *b–d*, Results of subsequent experiments (9–12 October 1987) in which the headspace of the same chamber was adjusted to initial CH_4 concentrations of 10, 50 and 550 p.p.m., respectively. Chamber temperatures differed from ambient temperatures by $<2^\circ\text{C}$ during the experiment.

FIG. 2 Soil characteristics and depth distribution of CH_4 consumption in a soil core. Maximum consumption rates occur in the top 5 cm of the core. No CH_4 was consumed by the vegetation. Water content ([wet weight/dry weight] - 1), organic content ([loss on ignition]/[dry weight]) and bulk density (ρ represents [oven-dried mass]/[field volume] in g cm^{-3}) are given for each of the soil units.



soils studied. In all cases, the concentrations eventually level off to a small but non-zero value. Figure 1 shows the results of an experiment on one of two chambers where atmospheric methane (~ 1.7 p.p.m.) was consumed, and subsequent experiments where the chamber headspace was adjusted to initial CH_4 concentrations of 10, 50 and 500 p.p.m. by injection of millilitre quantities of a 95% Ar:5% CH_4 mixture. Headspace concentrations in chambers with 10 p.p.m. CH_4 became sub-ambient in 4–7 h and decreased to 0.14 p.p.m. in as little as 8 h.

Soil cores from the same site were collected in plastic tubes (6.7-cm inner diameter, 2-mm wall) for laboratory studies of CH_4 oxidation and the depth distribution of CH_4 oxidizing potential. We avoided sample disturbance by cutting around the tube perimeters with a serrated knife as the tubes were inserted. The cores collected were 2–12 cm long, and showed a 2-cm fibric-hemic layer below the surface vegetation followed by a 3-cm dark organic-rich layer that graded into a weathered mineral horizon extending below the sample (Fig. 2). These cores were capped at the base and were placed in ~ 1 -l Mason jars, the lids of which were equipped with valves and a septum. The jar headspaces were maintained at constant pressure by introducing ambient air following sample removal; this headspace dilution was negligible. Because only the soil surface was in contact with the jar atmosphere, the cores in jars were physically equivalent to the field chambers. The ratios of headspace volume to soil surface area for the chambers and jars agreed to within 5%, so CH_4 uptake kinetics for both are comparable. The response of whole cores to CH_4 additions at ambient air temperatures was repeatable and identical to the field chambers—the first-order rate constants for methane consumption in both were $\sim -0.25 \text{ h}^{-1}$ and headspace methane concentrations as low as 0.7 p.p.m. were observed in 6–13 h.

Methane consumption by core segments representing the various soil horizons was measured in jar experiments using initial headspace CH_4 concentrations of ~ 10 p.p.m. Microlitre quantities of the same Ar: CH_4 mixture that was used in the chamber experiments were added to the jars. The results are shown in Fig. 2. The surface vegetation consumed no CH_4 . Methane was consumed by all soil layers, with the highest rates in the 0–2 cm and 2–5 cm layers. A portion of each core segment was autoclaved; these samples consumed no CH_4 in 24 h, indicating that CH_4 consumption is biologically mediated.

We performed a tracer experiment to determine the products of methane oxidation and their distribution in soils. Two cores with nearly identical methane uptake kinetics (cores C and E) were used in this experiment, one to follow CH_4 decreases and

the other to follow changes in headspace $^{14}\text{CH}_4$ and $^{14}\text{CO}_2$. The experiment was conducted in the dark to prevent photosynthetic uptake of $^{14}\text{CO}_2$. The headspaces of the jars containing both cores were adjusted to an initial CH_4 concentration of ~ 10 p.p.m., but stable CH_4 was added to core C whereas core E received CH_4 containing $^{14}\text{CH}_4$ (2.8×10^8 d.p.m. cm^{-3} ; d.p.m., disintegrations per minute). Core E was sampled at time intervals based on decreases in the stable CH_4 concentration of core C. Headspace samples (10 cm^3) were injected into a metal and glass stripping/oxidation line²⁷ and were carried in a helium flow through bubbler traps and a quartz combustion tube filled with CuO heated to 800 °C. The $^{14}\text{CO}_2$ was trapped in 1N NaOH before combustion and the $^{14}\text{CH}_4$ was trapped in Woeller's solution²⁸ as $^{14}\text{CO}_2$ following combustion. The jar headspaces were maintained at constant pressure by introducing ambient air following sample removal. The results are corrected for dilution (1.6% per sample). The experiment was terminated after 11 h when the CH_4 concentration reached 0.4 p.p.m. and 95% of the added $^{14}\text{CH}_4$ had been consumed. The remaining headspace $^{14}\text{CH}_4$ and $^{14}\text{CO}_2$ was recovered with a series of helium flushes; the jar and core were frozen and the ^{14}C incorporated in the core was assayed by dry combustion of freeze-dried homogenized samples of core segments.

Figure 3 shows the results of the tracer experiment. Core C showed a decrease in headspace CH_4 that parallels the $^{14}\text{CH}_4$ decrease in core E. The headspace $^{14}\text{CO}_2$ activity indicated that part of the consumed $^{14}\text{CH}_4$ was retained by the core. We accounted for 96% of the $^{14}\text{CH}_4$ added to core E; 46% of this was respired as $^{14}\text{CO}_2$ and 54% was recovered by dry combustion. The fraction recovered by dry combustion represents $^{14}\text{CH}_4$ assimilated into microbial biomass, organic matter and inorganic matter. We made no attempt to independently assay the inorganic fraction; these soils are carbonate-free and soil pH values of 5.7 suggest that this fraction is small.

Interpretation of experiments involving amended atmosphere requires an understanding of the timescale of equilibration between the soil and headspace for added gases. The equilibration time was evaluated for core E with a relaxation technique²⁹ that involved inhibiting methane oxidation and following equilibration of added CH_4 . Acetylene, a well-known inhibitor of CH_4 oxidation³⁰, was added to the headspace (30 p.p.m. final concentration) before sectioning core E for freeze-drying and dry combustion. Methane was then added and headspace concentrations were measured at 1-min intervals until the changes could not be resolved (6 min). This approach shows that equilibration is a two-step process: a fast ($k = -1.54 \text{ min}^{-1}$)

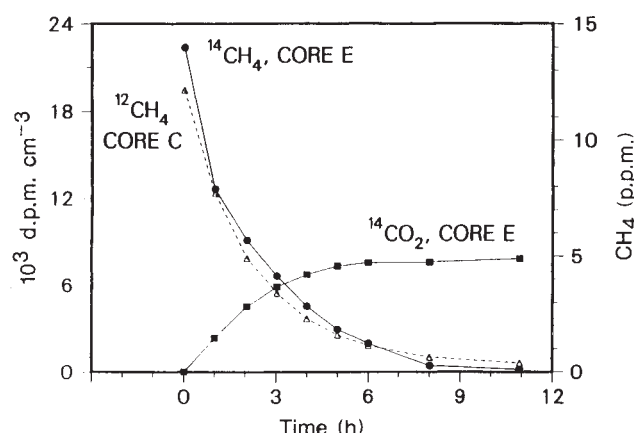


FIG. 3 $^{14}\text{CH}_4$ oxidation as a function of time. This jar experiment was conducted on 12 October 1987, using two cores with almost identical CH_4 oxidizing characteristics. The headspace of core C was amended with CH_4 and was used to guide sampling core E, which was amended with $^{14}\text{CH}_4$.

process, presumably involving the porous surface material or large voids, followed by a slower ($k = -0.75 \text{ min}^{-1}$) process involving more compact material or adjacent smaller voids. These first-order equilibration or relaxation constants show that equilibration is much faster than biological consumption ($k = -0.004 \text{ min}^{-1}$). Thus, CH_4 consumption is not controlled by transport in tundra soils. The initial samples in the chamber, jar and tracer experiments (taken 15 min after adjusting initial concentrations in the chamber and jar experiments; 5 min after tracer addition) contain no bias owing to incomplete equilibration.

The ability of these soils to respond to methane increases without a lag and to consume a wide (thousand-fold) range of concentrations with no indication of saturation (all follow first-order consumption kinetics) indicates that they could be an important sink. These methane consumption measurements were made at the low end (7°C) of the reported temperature range ($2\text{--}30^\circ\text{C}$). Methane consumption rates of $2.7 \text{ mg m}^{-2} \text{ d}^{-1}$ for our unamended chambers lie in the middle of reported ranges ($0.2\text{--}4.2 \text{ mg m}^{-2} \text{ d}^{-1}$)^{16–25}, indicating that methane oxidation can be important in cold soils. Final methane concentrations as low as those observed in our chamber and jar experiments ($0.1\text{--}0.4 \text{ p.p.m.}$) have not been reported in previous chamber experiments, but similar concentrations have been observed in soils¹⁶. Static chambers are rarely allowed to 'run down', so the high final concentrations in previous studies can be expected when static chambers are used for only short periods to avoid disturbing sub-surface gradients³¹. Our results demonstrate that soil oxidation of atmospheric CH_4 is microbially mediated and point to a population capable of oxidizing CH_4 at concentrations ten times lower than ambient atmospheric concentrations. Kinetic properties of cultured methanotrophs are not consistent with growth using atmospheric CH_4 as the sole carbon source, implying a threshold for growth well above atmospheric levels³². Mixotrophic growth, co-oxidation by non-methanotrophs such as ammonium-oxidizing bacteria, or induction of high-methane-affinity enzyme systems are processes that may be important in maintaining these low CH_4 concentrations^{30,32,33}.

These measurements have implications important to tundra systems under the projected warmer drier conditions. The sub-surface CH_4 oxidizing activity is important in controlling upward CH_4 fluxes at sites where vascular plants are absent. Vascular plants transport CH_4 and effectively bypass the CH_4 oxidizing zone. Tussock and low-shrub tundra (35–50% vascular plant cover) are expected to show larger consumption effects than wet meadow tundra (80–90% vascular plant cover)²⁶. Our previous observations at relatively wet permanent sites have not shown net oxidation²⁶. Methane fluxes from tundra³⁴ and swamp¹⁹ sites, however, are positively correlated with the level of the water table, and the 20% of the stations occupied during

a high-latitude transect study²⁴, all with a lowered water table, had zero or negative CH_4 fluxes.

Consumption of atmospheric CH_4 by soils depends on transport to zones of consuming activity. Transport in waterlogged tundra soils is by aqueous molecular diffusion. Waterlogged tundra sites have a methane consumption maximum centred near the water table (the oxic/anoxic boundary), sustained by upward diffusion of dissolved CH_4 . By contrast, transport in dry tundra soils is by gas-phase diffusion (10^5 times faster than aqueous), so porous low-resistance tundra soils do not limit the supply of methane. Expected consequences of atmospheric warming (such as a lowered water table, increased seasonal thaw depth, or permafrost melting) will increase the vertical extent of oxidized tundra soil and enhance oxidation of CH_4 . Our measurements demonstrate that the oxidation of atmospheric CH_4 occurs in oxic soils and is not limited by diffusion in non-waterlogged soils, so a negative feedback on atmospheric CH_4 concentration is possible. Furthermore, the CO_2 produced by oxidizing CH_4 is 20 times less effective as a greenhouse gas⁸. □

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Uranium and ^{10}Be enrichments by fluids in Andean arc magmas

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VOLCANIC rocks from subduction settings frequently contain ^{10}Be enrichments that have been attributed to sediment subduction¹⁻⁶, and are often characterized by $(^{238}\text{U}/^{230}\text{Th})$ ratios greater than unity, in contrast to mid-ocean-ridge and ocean-island basalts^{7,8}. Here we report such an excess of ^{238}U over ^{230}Th in historic lavas from the Southern Volcanic Zone of Chile (33–42° S). The greatest uranium excess is found south of 39° S, where $^{10}\text{Be}/^9\text{Be}$ ratios are highest and the trench is filled with sediment. These features suggest that both U and ^{10}Be enrichments result from preferential partitioning of U and Be into a fluid phase during dehydration of the recently subducted sediments. The presence of strong ^{238}U – ^{230}Th disequilibria in basaltic magmas of the Southern Volcanic Zone suggests that the timescale for dehydration, melting and eruption in this arc is probably less than ~20,000 yr.

Andean Southern Volcanic Zone (SVZ), where subduction-related volcanism has occurred continuously since the Jurassic, is a single arc segment underlain by continental crust (Fig. 1). North of 37° S, the crust ranges in age from late Palaeozoic to Triassic and its thickness increases sharply from 35 km to 55–60 km between 34 and 36° S (ref. 9). South of 37° S, the crust is of Mesozoic to Cenozoic age and has a relatively uniform thickness of 30–35 km until 40° S where it thickens slightly to ~40 km (ref. 10). Throughout the SVZ the geometry and rate

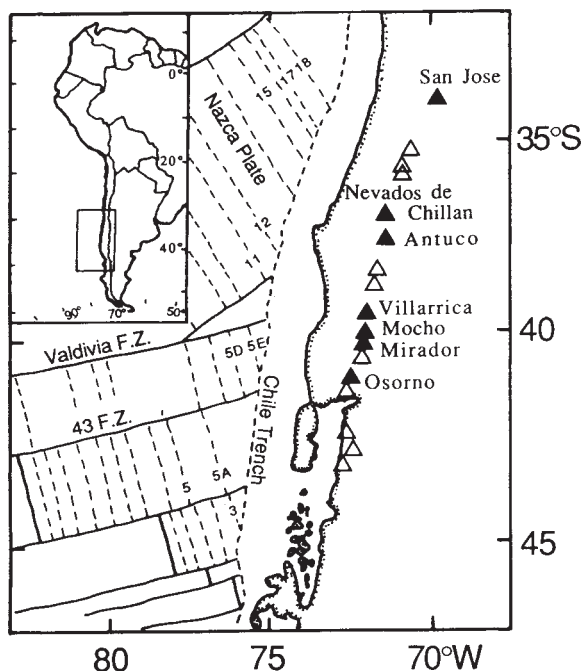


FIG. 1 Map of a part of the Southern Volcanic Zone of the Andes showing the volcanoes discussed. Nazca plate features are taken from ref. 11.

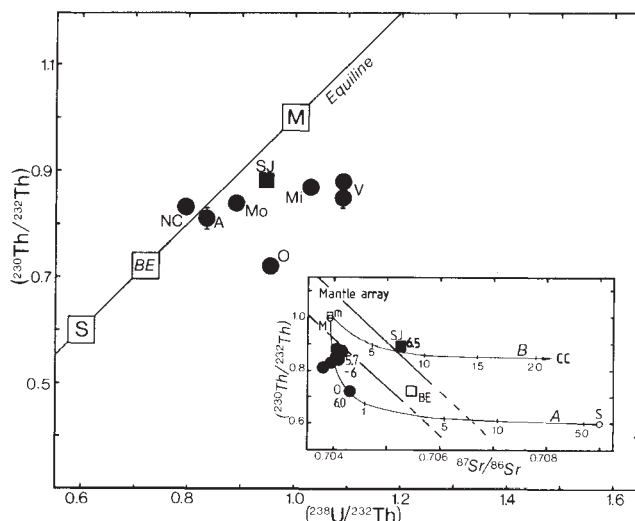


FIG. 2 Data for the SVZ volcanoes in the $(^{230}\text{Th}/^{232}\text{Th})$ – $(^{238}\text{U}/^{232}\text{Th})$ isochron diagram. Samples in U–Th radioactive equilibrium would plot on the equiline. NC, Nevados de Chillan; A, Antuco; Mo, Mocho; SJ, San Jose; O, Osorno; Mi, Mirador; V, Villarrica. BE is the 'Bulk Earth' value¹⁸, M, mantle of ocean-island-basalt (OIB) type; S, sedimentary component. The SVZ mantle source on the equiline can have been modified by addition of ancient sediments to an OIB-like mantle. Inset, Th–Sr isotope diagram of the same samples. The numbers adjacent to data points indicate the $\delta^{18}\text{O}$ values of the samples. Curve A: Hypothetical model explaining the modification of the mantle sources under the SVZ by mixing between an OIB-like mantle source (M), with $(^{230}\text{Th}/^{232}\text{Th})=1.0$, $^{87}\text{Sr}/^{86}\text{Sr}=0.7039$, Th=0.04 p.p.m., Sr=20 p.p.m., and a sedimentary component (S) with $(^{230}\text{Th}/^{232}\text{Th})=0.6$ (inferred from Th/U ratio), $^{87}\text{Sr}/^{86}\text{Sr}=0.709$, Th=15 p.p.m., and Sr=300 p.p.m. (from ref. 19). Curve B: Crustal contamination model for the San Jose magma: A mantle melt (m), having $(^{230}\text{Th}/^{232}\text{Th})=1.0$, $^{87}\text{Sr}/^{86}\text{Sr}=0.7039$, Th=0.5 p.p.m., Sr=400 p.p.m., assimilates Palaeozoic–Triassic crustal rocks (CC) with $(^{230}\text{Th}/^{232}\text{Th})=0.8$, $^{87}\text{Sr}/^{86}\text{Sr}=0.770$, Th=10 p.p.m., and Sr=100 p.p.m. (from ref. 9). The numbers on curves A and B refer to the percentage of contamination by sediments and continental crust, respectively.

of subduction and the distance of the volcanic front from the trench remain nearly constant, although in the northern part between 33–37° S the age of the subducting crust is ~40 Myr and the trench is sediment-poor, whereas in the southern part between 37 and 42° S the age of the subducting crust is ~18 Myr and the trench is filled with sediment¹¹. The absence of a significant accretionary prism inboard of the trench indicates that sediments are subducted beneath the arc¹².

Our samples are lavas from eight historic eruptions at seven volcanic centres between 33 and 42° S (Fig. 1). The samples are calc-alkaline, high Al basalts and basaltic andesites, with two andesites and a dacite included in the suite. Only one volcano, San Jose, is situated to the north of 37° S and, therefore, has been constructed on a thicker and older crust than the other volcanoes. For the six volcanic centres south of 37° S, medium-K lavas consist of anhydrous mineral assemblages composed predominantly of olivine and plagioclase, with occasional clinopyroxene in mafic rocks, and plagioclase and two pyroxenes in intermediate lavas, whereas the lava from San Jose is a high-K hornblende andesite. The analytical results of ^{238}U – ^{230}Th disequilibria and Sr and O isotopes together with previous ^{10}Be measurements² are reported in Table 1.

When plotted on the isochron diagram (Fig. 2) the SVZ data form a nearly horizontal array extending right from the equiline. The $(^{238}\text{U}/^{230}\text{Th})$ ratios range from 0.96 to 1.33, whereas $(^{230}\text{Th}/^{232}\text{Th})$ ratios vary only between 0.81 to 0.88, with the exception of the Osorno basalt, which has the lowest measured $(^{230}\text{Th}/^{232}\text{Th})$ ratio of 0.72. Intermediate and evolved lavas are similar to mafic ones in terms of $(^{230}\text{Th}/^{232}\text{Th})$ ratios. Despite

TABLE 1 Isotope data

Latitude	Volcano	Date of eruption	Rock type	SiO ₂ (wt%)	U (p.p.m.)	Th (p.p.m.)	Th/U	$\left(\frac{^{238}\text{U}}{^{232}\text{Th}}\right)$	$\left(\frac{^{230}\text{Th}}{^{232}\text{Th}}\right)$	$\left(\frac{^{238}\text{U}}{^{230}\text{Th}}\right)$	^{10}Be ($\times 10^6$ atom g ⁻¹)	$^{10}\text{Be}/^9\text{Be}$ ($\times 10^{-11}$)	$^{87}\text{Sr}/^{86}\text{Sr}$	$\delta^{18}\text{O}$ (‰SMOW)
33°45' S	San Jose, 281284-4	1800s	And	62.5	3.24	10.4	3.21	0.945	0.88±0.01	1.07	1.4	1.0	0.70527±2	6.5
36°45' S	N Chillan, 060376-1	1930s	Dac	65.6	2.29	8.73	3.82	0.795	0.83±0.01	0.96	1.8	1.4	0.70395±2	5.9
37°22' S	Antuco, 051177-7	1853	Bas	50.2	0.402	1.46	3.64	0.834	0.81±0.02	1.03	1.0	2.3	0.70379±2	5.9
39°25' S	Villarrica, 101275-1	1971	Bas	52.5	0.447	1.24	2.78	1.09	0.85±0.02	1.28	2.1	5.3	0.70404±2	5.8
39°25' S	Villarrica, 191284-1	1984	Bas	52.3	0.429	1.19	2.77	1.09	0.88±0.01	1.24			0.70403±2	5.7
40°50' S	Mocho, 220281-1	1864	And	58.8	0.873	2.98	3.41	0.889	0.84±0.01	1.06	1.3	2.0	0.70410±2	6.0
40°30' S	Mirador, 220479-3	1979	Bas	53.5	0.236	0.697	2.95	1.03	0.87±0.01	1.19			0.70413±2	5.9
41°10' S	Osorno, 090185-1H	1834	Bas	53.0	0.303	0.963	3.18	0.954	0.72±0.01	1.33	1.4	3.7	0.70429±2	6.0

U and Th contents are determined by isotope-dilution mass spectrometry. Ratios in parenthesis are activity ratios. The relative 1 σ errors on ($^{238}\text{U}/^{232}\text{Th}$) and ($^{230}\text{Th}/^{232}\text{Th}$) ratios are estimated at 0.5% and 2% respectively. The errors on ($^{230}\text{Th}/^{232}\text{Th}$) are 1 σ statistical α -counting errors. Reproducibilities (1 σ) for measurements are better than $\pm 20\%$ for $^{10}\text{Be}/^9\text{Be}$ ratio. The Sr isotope ratios have been normalized to a value of $^{86}\text{Sr}/^{88}\text{Sr}=0.1194$ and $^{87}\text{Sr}/^{86}\text{Sr}=0.71022$ for the NBS-987 standard; the errors are 2 σ in-run errors. Reproducibilities for $\delta^{18}\text{O}$ are better than 0.2‰ and the NBS-28 gave $\delta^{18}\text{O}=9.6\%$ ($\delta^{18}\text{O}=[(^{18}\text{O}/^{16}\text{O})_{\text{sample}}/(^{18}\text{O}/^{16}\text{O})_{\text{SMOW}}]-1$, SMOW is standard mean ocean water). wt%, weight per cent.

the near constancy of Th isotope ratios, the Th/U ratios vary considerably (2.77–3.82) but with no clear geographical trend along the strike of the arc.

The ($^{230}\text{Th}/^{232}\text{Th}$) ratios of SVZ lavas are too low to be inherited from a mantle source such as those giving rise to mid-ocean-ridge basalt (MORB) or ocean island basalt (OIB), yet the similarity of the ($^{230}\text{Th}/^{232}\text{Th}$) ratios of most SVZ lavas favours an origin from a rather homogeneous reservoir. Crustal contamination would hardly result in the constant ($^{230}\text{Th}/^{232}\text{Th}$) ratios in different lava types from distant volcanoes. The low Th isotope ratios also preclude any major involvement of altered basalts from the subducted oceanic crust. Such basalts would be enriched in uranium and therefore would have high ($^{230}\text{Th}/^{232}\text{Th}$) ratios after radioactive equilibrium had been reached. The low ($^{230}\text{Th}/^{232}\text{Th}$) may be a characteristic of a subduction-modified mantle wedge underlying most part of the studied area. As sediments have been subducted since the Jurassic, the mantle sources have probably been affected by mixing with this component. Pelagic and terrigenous sediments often have high Th/U, and hence low ($^{230}\text{Th}/^{232}\text{Th}$) ratios (see Fig. 2). Thus sediment injection may have lowered the ($^{230}\text{Th}/^{232}\text{Th}$) of the sub-arc mantle, and this can explain the location of most data below the 'mantle array' in the Th–Sr isotope diagram (Fig. 2, inset). Mixing of a sedimentary component in the mantle source will also lead to higher $^{87}\text{Sr}/^{86}\text{Sr}$ ratios as schematically illustrated by the mixing curve. Most samples from 36 to 42° S have $^{87}\text{Sr}/^{86}\text{Sr}$ ratios around 0.7040, except for the basalt from Osorno, which has a slightly higher $^{87}\text{Sr}/^{86}\text{Sr}$ ratio and lower ($^{230}\text{Th}/^{232}\text{Th}$) ratio. Only a small contribution (<1%) of the sedimentary component is necessary to explain the thorium and strontium isotope data. Thus the $\delta^{18}\text{O}$ values will not be significantly affected in this process: they range from 5.7 to 6.0‰ and do not differ from normal mantle values. Only the high-K andesite from San Jose, north of 37° S, has higher $\delta^{18}\text{O}$ (6.5‰) and $^{87}\text{Sr}/^{86}\text{Sr}$ (0.7053): the magma in

this volcano has probably been contaminated by old and ^{18}O -rich continental crust (Fig. 2, insert).

Previous results indicate that the relatively constant Pb isotope ratios in the SVZ lavas^{9,10} can be explained neither by simple mixing of mantle material and presently subducted sediments, nor by a contamination from the continental crust⁹. But the introduction into the mantle of old and radiogenic sediments during the 200 Myr of convergence in this zone can produce the Pb isotope signature of the SVZ lavas. Radiogenic sediments could have been formed by erosion of the South America Precambrian craton and deposited by the rivers in the Pacific Ocean before the Andean orogeny. Another possible mechanism is the erosion of the Palaeozoic/Precambrian continental edge¹². Thus the Pb isotope data does not contradict the model of a mantle source progressively modified by subducted sediments.

The limited variation of the different isotope ratios in the SVZ lavas suggest that this contribution is relatively constant between 37 and 41° S and that the mantle wedge is well mixed and isotopically homogeneous. Further south, however, Osorno has a distinctive Th and Sr isotope composition, indicating a greater contamination of its mantle source by ancient sediments, perhaps related to a thinner mantle wedge in this area.

These time-integrated modifications of the mantle wedge do not explain the origin of the ^{238}U enrichment over ^{230}Th , which obviously requires a recent (<300,000 yr) event in the mantle source. This uranium enrichment is not restricted to oceanic island arcs as previously suggested^{8,13}. The fact that many subduction zone volcanoes lie on the right of the equiline in the isochron diagram is usually interpreted as reflecting the preferential uranium enrichment of the mantle wedge by slab-derived fluids before or during melting^{14,15}. In this context it is particularly appropriate to compare ^{238}U – ^{230}Th disequilibria and ^{10}Be results.

The presence of cosmogenic ^{10}Be in many lavas from young volcanic arcs and its total absence in MORB or OIB lavas

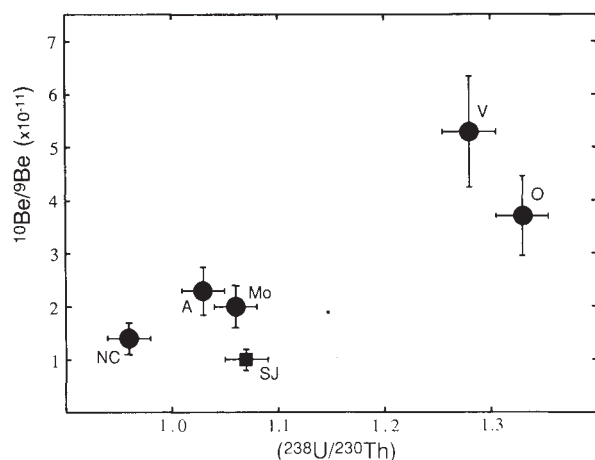


FIG. 3 Variation of $^{10}\text{Be}/^9\text{Be}$ with ($^{238}\text{U}/^{230}\text{Th}$) in the SVZ lavas. Abbreviations as in Fig. 2. Assuming a ^{10}Be -free mantle source, the extrapolation of this correlation to $^{10}\text{Be}/^9\text{Be}=0$ can give the ($^{238}\text{U}/^{230}\text{Th}$) ratio of the primary melt before fluid addition. If this value is constant for all the SVZ primary magmas, then the maximum proportion of U added by fluids to the primary melt can be calculated for each volcano; it amounts to $\sim 35\%$ for Villarrica.

supports the proposition that subducted pelagic sediments, younger than 10 Myr, contribute to the formation of arc magmas¹⁻⁵. Concentrations of ¹⁰Be for the historic SVZ lavas analysed are given in Table 1 and these values ($1.0\text{--}2.1 \times 10^6$ atom g⁻¹) are significantly higher than mantle or lower crustal values ($\ll 1 \times 10^6$ atom g⁻¹). They have been interpreted to reflect the incorporation of a subducted component during or before magma generation^{2,5,6}. The ¹⁰Be and (²³⁸U/²³⁰Th) ratios in SVZ lavas are compared in Fig. 3. Despite rather large analytical uncertainties in the ¹⁰Be measurements, a fair correlation is apparent; the lavas having the highest uranium enrichment (south of 39° S) also exhibit the highest ¹⁰Be/⁹Be ratios. These results are in contrast to those from the New Britain arc^{16,17}, where highest ¹⁰Be/⁹Be are associated with highest (²³⁰Th/²³²Th) ratios, but are not well correlated with uranium excesses. The correlation between ¹⁰Be/⁹Be and (²³⁸U/²³⁰Th) in the SVZ samples is even better if we exclude the contaminated San Jose sample. The high ¹⁰Be/⁹Be ratios of the Villarrica and Osorno lavas can be attributed to the injection in the mantle of a greater amount of recent sediments. Indeed the extension towards the continent of the Valdivia Fracture Zone and 43° Fracture Zone (Fig. 1) which may act as sediment traps, pass directly beneath these volcanic centres. The absence of any correlation between Pb isotopes^{9,10} and ¹⁰Be/⁹Be ratios confirms that, in contrast with ¹⁰Be/⁹Be ratios, the arc lead budget is the result of long-term modification of the mantle source by sediment subduction.

The correlation between ¹⁰Be/⁹Be and (²³⁸U/²³⁰Th) strongly suggests that a common process is responsible for the U and ¹⁰Be enrichment. Boron probably accompanies ¹⁰Be and U because ¹⁰Be/⁹Be and B/⁹Be ratios are well correlated for SVZ lavas⁶. This process is most probably the recent dehydration of subducted sediments. The correlation also suggests that the U and ¹⁰Be enrichments are related to the abundance of fluids and, therefore, to the amount of recently subducted sediments. The sediment subduction does not inhibit large earthquakes as evidenced by the great 1960 earthquake, which had its epicentre in this region of the Andes.

If the U and ¹⁰Be enrichment is indeed the result of fluid transfer to the zone of magma generation in an otherwise homogeneous mantle source, then the nearly horizontal array in the isochron diagram (Fig. 2) has an age significance: it shows that the time interval between the fluid induced U-Th fractionation in the mantle and eruption of the magmas is $\leq 20,000$ yr. Further discussion on the relative contributions of old and recent subducted sediments, mantle wedge and continental crust requires further data on other SVZ volcanoes, but our study demonstrates that U and ¹⁰Be enrichments in the SVZ magmas are produced by the introduction in the mantle of fluids derived from sediments presently subducted under the Andes. □

Observations of seismic reflectors in the lower lithosphere beneath the Skagerrak

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THE lithosphere—the rigid outer shell of the Earth—includes both the crust and the upper mantle. In the past decade, the complex continental crust and underlying shallower mantle lithosphere have been extensively studied using reflection seismology, but the lower lithosphere has not been successfully imaged. Here we report the observation of continuous and virtually horizontal reflectors in the lower continental lithosphere down to depths of 100–110 km below the Skagerrak (Scandinavia). These are apparently not structurally associated with the crust, whereas previously reported mantle reflectors, such as those observed by the BIRPS group around the British Isles^{1,2}, are often located near large faults in the overlying crust. Like the crust, the mantle lithosphere shows a seismically transparent upper and a reflective lower part. Thus, the processes that form seismic reflectors seem to have an affinity for ductile rheological environments in the mantle as well as in the crust. Our observations are consistent with a model consisting of a mechanically strong upper lithosphere, underlain by a thermal boundary layer which separates it from the convecting asthenosphere.

The roughly horizontal mantle reflectors were recorded on a 100-km-long experimental ultra-deep seismic reflection profile (OG-13) that was part of a comprehensive deep seismic survey (1,730 km; 16-s record length) conducted by the research vessel *Mobil Search* in the Skagerrak in 1987 (Fig. 1)³. A 40-s record length was used with a 4.5-km-long streamer having 180 hydrophone groups. The energy source was a 9,000-in³ airgun array. As reflected energy is low for the large depths involved, much effort was put into processing refinements. After extensive experimentation, we chose a BIRPS processing scheme⁴ except that we used a weighted trace mix in the common offset domain before stacking. The latter process gave a significant improvement of the data quality in the lower parts of the section as compared to more conventional extended-array simulations.

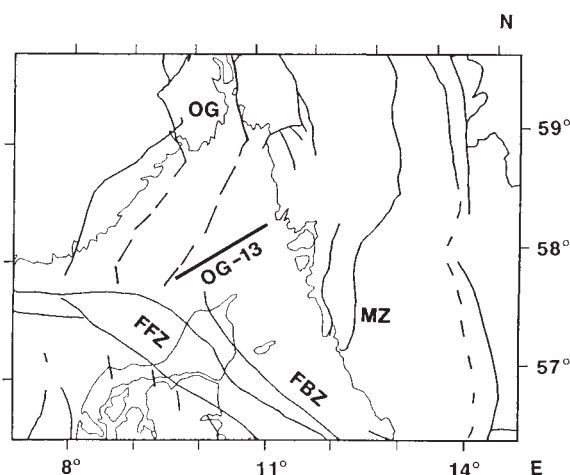


FIG. 1 Map showing the location of the deep seismic profiling line OG-13 in the Skagerrak. The principal geological features are outlined; OG: Oslo Graben; FBZ: Fennoscandian Border Zone; FFZ: Fjerritslev Fault Zone; MZ: Mylonite Zone.

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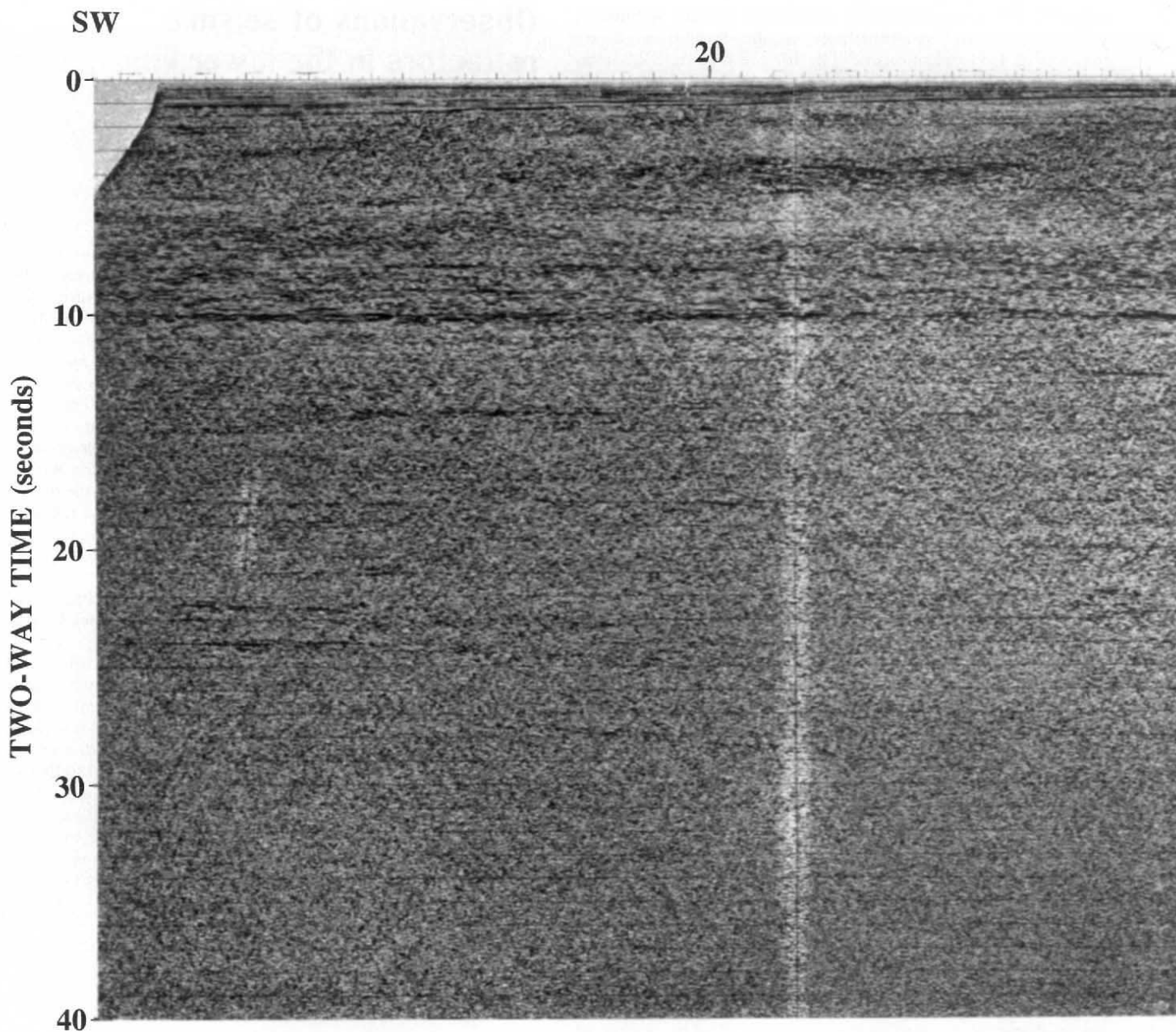


FIG. 2 Unmigrated seismic section of the southwest part of the OG-13 profile. Shot-point separation is 100 m, vertical-axis record length is two-way travel time (s).

Figure 2 is a stacked section and Fig. 3 a line drawing of the most prominent reflectors along OG-13. From Fig. 2 we note that just below the layer of sediments, which becomes thinner in the northeast, crustal reflectors are generally absent. However, patches showing a different character seem to outline structural bodies in the upper crust. At mid-crustal levels there is an increase in reflectivity, which may mark the transition from an brittle upper to a ductile lower crust^{5,6}. Lower crustal reflectors with individual lengths of a few kilometres often form lineaments 20–30 km long. The crustal reflectors seem to exhibit a distinct pattern that dips and thins towards the northeast where the crust thickens towards the centre of the Fennoscandian Shield. The reflective lower crust is bounded below by the 'reflection' Moho, which is well defined as an almost continuous reflective band ~200–400 ms (0.7–1.5 km) wide. Closer inspection reveals that the Moho at 10-s two-way travel time (~30 km) is laminated with 3–6 parallel segments, each typically 1–4 km long. Below

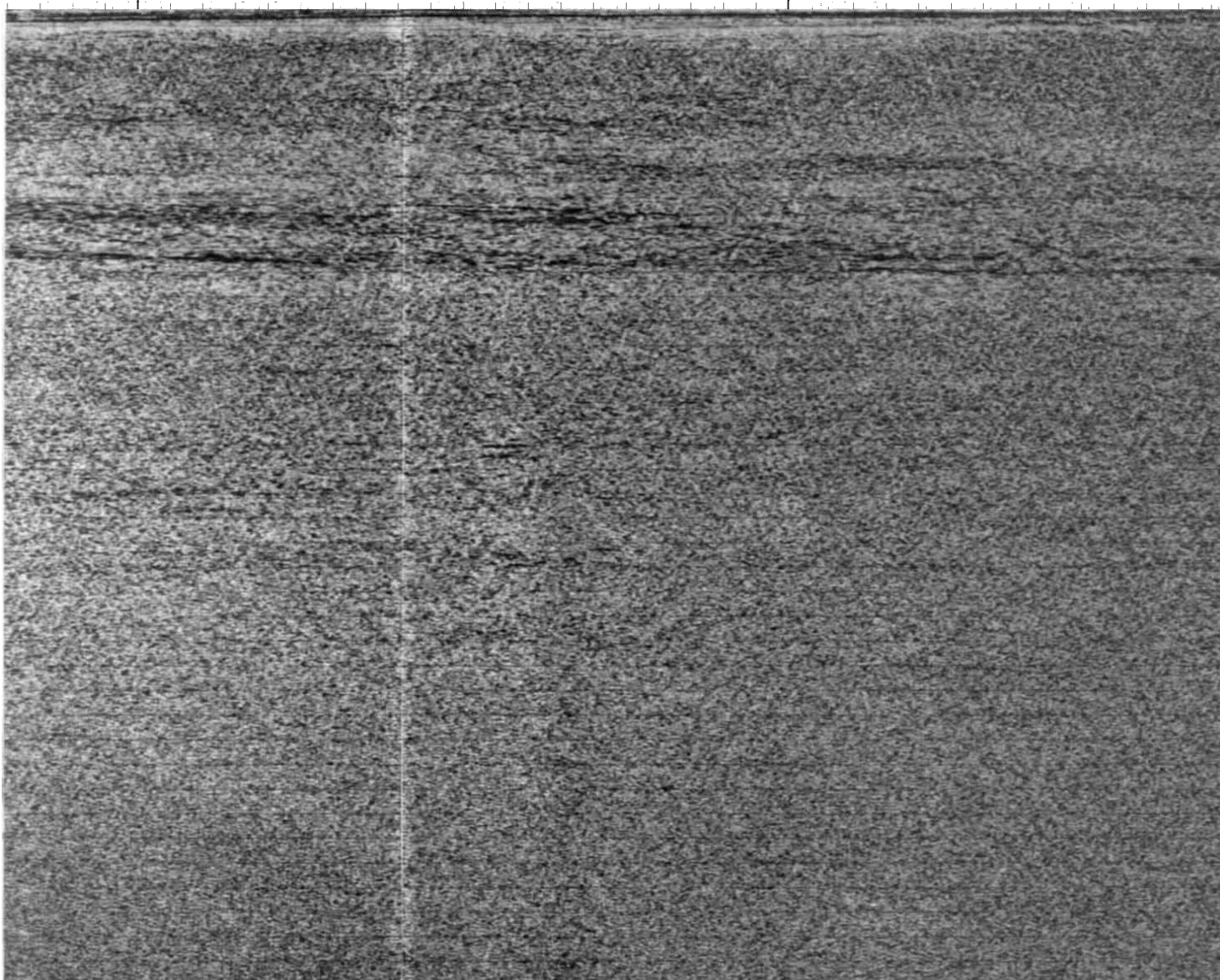
the Moho, reflectivity decreases abruptly, but reappears again at ≥ 14 s (50 km) in the seismic section. The individual reflectors have lengths up to 40 km, but most lie in the 5–20 km range. Their vertical separation often exceeds 10 km, and the deepest reflectors are observed at a two-way travel time of ~29 s, which here is equivalent to a depth of 100–110 km. We note that reflectors are relatively sparse in the northeastern part of the profile, and that a similar weakening of reflections from the Moho is also observed. We attribute these phenomena to scattering of the seismic energy by the hard and uneven sea floor in this part of the section, where a sediment cover is lacking. In the western half of the section a possible candidate for a converted S-wave reflection from the Moho is observed and has been modelled to have an arrival time of 14 s. We note, however, that a similar observation of a potential S-wave converted reflection from the Moho, the W-reflector on the DRUM profile off northwest Scotland¹, was later proved to be a P-wave reflection and not an S-wave artefact by the SLAVE two-ship experiment⁷.

The existence of reflectors 5–20 km long in the lower mantle lithosphere have rarely been reported. Notable exceptions are the MOIST and DRUM profiles off northeast Scotland^{1,8} and

NE
km

40

60



the Pannonian geotraverse⁹. None of the previously reported examples, however, show such continuous horizontal reflectors near the probable base of the lithosphere.

The reflectivity-depth distribution in the mantle seems to be similar to that observed in the crust, with reflectors increasing in abundance below a certain depth. As both the crust and the mantle lithosphere probably consist of a brittle part overlying a more ductile layer¹⁰, this supports a model of preferential reflector formation in a ductile environment. Among the possible explanations that have been put forward for the large impedance contrasts needed to form mantle reflectors are the presence of shear zones containing layers of hydrated minerals, caused by the passage of water, and layers of mafic igneous material due to partial melting¹. Alternatively the presence of shear strain may favour layers with oriented olivine or large amounts of garnet^{11,12}. Such hypotheses have not yet been substantiated by quantitative modelling, however, and no objective criteria exist to discriminate amongst them using seismic data. As the nature of mantle reflections remains an enigma, we refrain from further speculations on their character in the Skagerrak area.

The deepest reflections identified, at ~100–110 km depth, are

near the base of the lithosphere under this part of Scandinavia, as estimated from Rayleigh-wave analysis, 110–120 km (ref. 13). Lithosphere cooling, as defined by tectonic subsidence using the backstripping method in the area immediately west of the Fennoscandian Border Zone (Fig. 1) gives a thermal lithosphere thickness there of ~125 km (ref. 14). The somewhat thicker thermal lithosphere may be due to the presence of a 'thermal boundary layer' separating the upper mechanical part of the lithosphere from the underlying convecting asthenosphere¹⁵. Whereas the mechanical part is capable of preserving structural and compositional features on a geological timescale, these are smeared out in the thermal boundary layer owing to episodic convection. Consequently, we do not expect to observe any reflectors there. It is also possible that the lack of observed reflectors is related to the limited dynamic range of the recording system and limited strength of the airgun signal, but it seems unlikely that the 'penetration depth' so defined should agree so well with lithosphere thickness as estimated by other and independent methods.

Our results indicate that the base of the lithosphere can be mapped using the seismic-reflection technique. Such results,

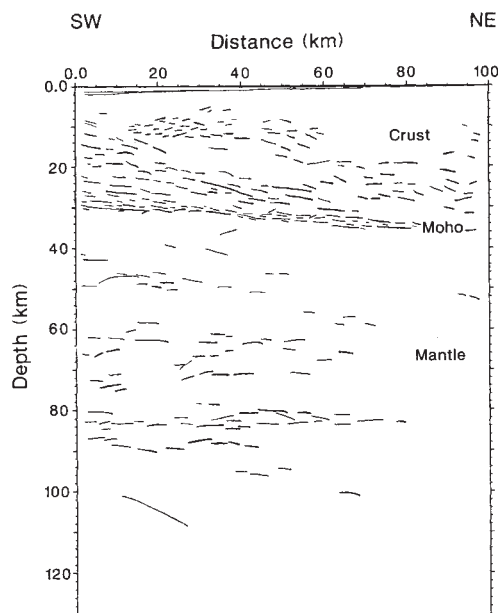


FIG. 3 Line drawing derived from the time-to-depth conversion of the unmigrated stack section using a simple velocity model.

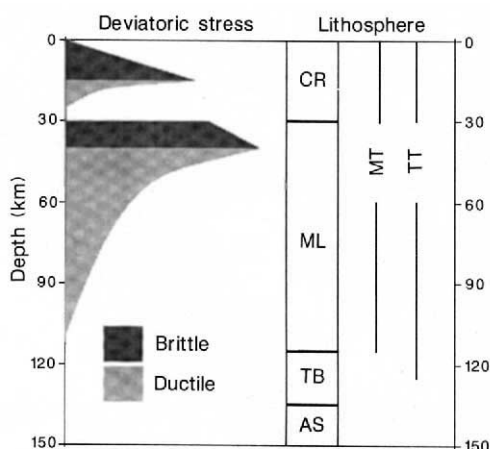


FIG. 4 Sketch of the lithosphere in the OG-profiling area. CR, crust; ML, lower lithosphere; TB, thermal boundary layer; AS, asthenosphere; MT, mechanical lithospheric thickness; TT, thermal lithospheric thickness. Deviatoric stress (relative scaling) calculated for a lithosphere rheology with quartz in the crust and olivine in the mantle lithosphere. The brittle and ductile regions are indicated. The thermally defined lithosphere is thicker than the one estimated from surface-wave analysis owing to the presence of the thermal boundary layer.

combined with data from other geophysical methods, may ultimately help us to improve our understanding of the nature of the lithosphere, its transition to the asthenosphere and the part it plays in plate tectonics. □

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Liquid immiscibility in a nephelinite–carbonate system at 25 kbar and implications for carbonatite origin

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MANTLE-DERIVED carbonate-rich melts may have an important role in mantle metasomatism^{1,2}, and may serve as parent liquids for crustal carbonatite magmas^{3,4}. Experiments have shown that carbonatitic melts can be produced by partial melting of peridotite + CO₂ + H₂O above 22 kbar (ref. 3), and that silicate and carbonate liquids are immiscible between 2 and 15 kbar for a wide range of Ca/Na ratios^{5–7}. We have determined the extent of silicate–carbonate liquid immiscibility at 25 kbar and 1,050–1,300 °C using mixtures of magnesian nephelinite, dolomite and sodium carbonate with and without water. In contrast to the low-pressure data, the two-liquid field at 25 kbar is restricted to more sodium-rich compositions, far removed from natural mantle melts. Our experimental results suggest that neither partial melting of carbonated peridotite, nor extensive fractional crystallization of silicate magmas at depths corresponding to 25 kbar, are likely to generate carbonatitic magmas by liquid immiscibility.

High-pressure melting studies in the system CaO–MgO–SiO₂–CO₂ have shown that initial liquids will be dominated by calcic dolomite (Ca/Mg > 1) and will have ~5–10 wt% silicate components^{8,9}. In natural peridotites sodium will be strongly partitioned into this carbonatitic liquid^{3,10}. None of these high-pressure melting studies, however, has demonstrated silicate–carbonate liquid immiscibility. Mixtures of kimberlite and calcite with and without H₂O did not show liquid immiscibility over a temperature and pressure range of 1,250–1,500 °C and 20–30 kbar (ref. 11). In vapour-saturated peridotite melting experiments above 31 kbar, Wallace and Green³ reported possible silicate–carbonate liquid immiscibility at ~1,000 °C. Our study was undertaken to define the extent of silicate–carbonate liquid immiscibility over a range of temperatures and compositions at 25 kbar. Starting mixtures contained 30–50 wt% of a de-watered primitive nephelinite (Neph) from the Honolulu Volcanic field, Hawaii (68KEE-1)¹². The remaining 50–70 wt% consisted of carbonate with ratios of dolomite (Dol) and Na₂CO₃ between 0 and 3. Mixtures with the same major-element compositions and containing 4.5 wt% H₂O were made up from combinations of oxides, carbonates and synthetic and natural minerals. Water was added in the form of Mg(OH)₂ and Al(OH)₃; 4.5 wt% was selected as a reasonable upper limit for mantle melts. A mixture consisting of 44 wt% nephelinite, 6 wt% Durango apatite and 50 wt% Na₂CO₃ was used to investigate the effects of P₂O₅ on immiscibility. All starting mixtures were dried for at least 1 h in a vacuum drying oven at 110 °C before being loaded into graphite crucibles, which were sealed inside platinum capsules. Although not rigorously buffered, experimental calibration sug-

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TABLE 1 Coexisting liquids from selected 25-kbar experiments

Run T(°C) t(h)	116 1,250 10.2	110 1,150 9.2	157 1,050 6.0			
	Carb(10)*	Sil(9)	Carb(9)	Sil(11)	Carb(9)	Sil(9)
SiO ₂	9.5 (1)†	33.7 (1)	6.64 (5)	34.2 (1)	19.5 (2)	30.9 (1)
TiO ₂	1.02 (3)	2.42 (3)	0.68 (5)	2.68 (3)	1.49 (4)	2.07 (2)
Al ₂ O ₃	2.1 (1)	9.39 (5)	2.4 (1)	10.4 (2)	6.1 (1)	10.33 (4)
FeO	6.15 (8)	10.36 (6)	5.04 (7)	10.3 (1)	7.4 (2)	8.12 (8)
MgO	6.60 (7)	7.5 (1)	3.2 (1)	5.21 (8)	7.3 (2)	6.88 (7)
CaO	11.98 (9)	6.00 (6)	17.78 (8)	8.21 (9)	9.4 (1)	5.91 (4)
Na ₂ O	60.6 (3)	29.8 (2)	55.8 (3)	26.7 (2)	47.5 (4)	34.6 (2)
K ₂ O	1.03 (2)	0.43 (2)	0.82 (3)	0.35 (2)	0.62 (2)	0.74 (2)
P ₂ O ₅	1.09 (3)	0.30 (3)	7.32 (6)	1.92 (4)	0.65 (3)	0.41 (3)

* Number of analyses.

† Values in parentheses are standard deviations of the mean, that is, 9.5(1) means 9.5 ± 0.1 .

All liquids reported on a CO₂-free basis, normalized to 100%. Bulk compositions: 116 = 0.5Neph + 0.5Na₂CO₃; 110 = 0.44Neph + 0.06Ap + 0.5Na₂CO₃; 157 = 0.5Neph + 0.5Na₂CO₃ + 4.5% H₂O. Abbreviations: Carb, carbonate liquid; Sil, silicate liquid; Neph, nephelinite; Ap, apatite.

gests that the oxygen fugacity in the graphite capsules was in the wüstite field¹³.

Experiments were performed in a 1.27-cm piston-cylinder apparatus using calcium fluoride as a pressure medium. For the anhydrous starting compositions a series of timed experiments showed that a minimum of 6–8 h was required to approach two-liquid equilibrium. The hydrous experiments were run for 1–6 h. Products were polished without using water, and were analysed with a Camscan scanning electron microscope (SEM) fitted with an energy dispersive system. Quenched liquids and crystals were analysed using a beam current of 0.1 nA on brass. All of the liquids quenched to a fine intergrowth of carbonate and silicate material. The quenched liquids were analysed 9–11 times in raster mode; each analysis covered several hundred square micrometres. Liquid compositions from selected experiments are reported in Table 1.

Figure 1a shows the results of the 25-kbar nominally anhydrous experiments on the nephelinite–dolomite–sodium carbon-

ate join. Liquids and starting compositions have been projected from CO₂ onto the plane (SiO₂ + Al₂O₃ + TiO₂)–(CaO + MgO + FeO*)–(Na₂O + K₂O). This simple oxide wt% projection has been used extensively to display silicate and carbonate liquids at low to moderate pressures⁶. Also shown in Fig. 1a are the coexisting silicate and carbonate liquids in the systems sanidine–K₂CO₃ (ref. 14) and albite–Na₂CO₃ (D. Ellis and P.J.W., unpublished data), and the 5-kbar immiscibility field⁶. At 1,300 and 1,250 °C the two-liquid field is located in the sodium-rich portion of composition space. For both temperatures, the immiscibility field is broadest for mixture 1 (Dol/Na₂CO₃ = 0) and the field closes with increasing dolomite content. The field also widens with decreasing temperature.

At 1,250 °C both the mixture 1 (Dol/Na₂CO₃ = 0) and mixture 2 (Dol/Na₂CO₃ = 0.33) form two liquids. In both charges the silicate fraction forms a large spheroid (<800 μm in longest dimension) and is surrounded by carbonate melt. At 1,250 °C the silicate fraction in the mixture 1 experiment contains olivine

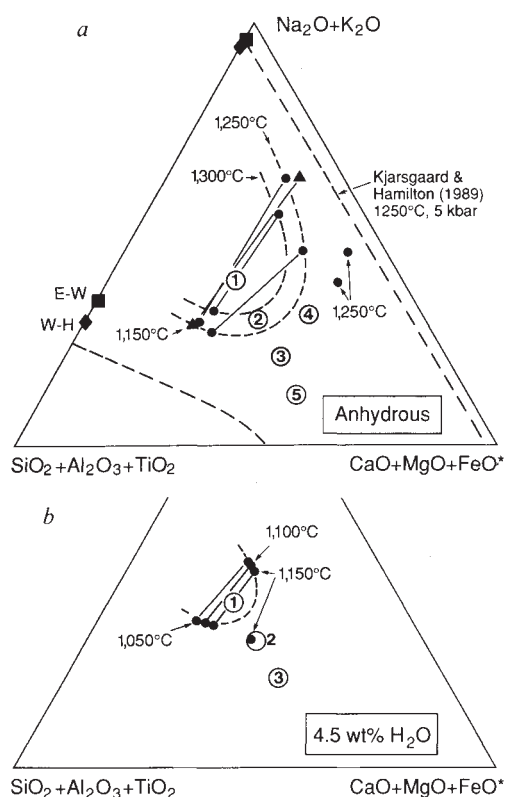


FIG. 1 Run products and starting compositions projected from CO₂ + H₂O onto the plane (SiO₂ + Al₂O₃ + TiO₂)–(CaO + MgO + FeO*)–(Na₂O + K₂O). FeO* is total Fe as FeO. Open circles denote starting mixtures: 1, 0.5Neph + 0.5Na₂CO₃; 2, 0.5Neph + 0.375Na₂CO₃ + 0.125Dol; 3, 0.5Neph + 0.25Na₂CO₃ + 0.25Dol; 4, 0.3Neph + 0.35Na₂CO₃ + 0.35Dol; 5, 0.5Neph + 0.125Na₂CO₃ + 0.375Dol; abbreviations as in Table 1 and Dol, dolomite. a, Anhydrous experiments; closed symbols denote liquid compositions, triangles denote liquids from mixture 1 + 6% apatite experiment at 1,150 °C. Long dashes denote 5-kbar two-liquid field from Kjarsgaard and Hamilton⁶; W–H denotes experimental liquid from Wendlandt and Harrison¹⁴; E–W denotes experimental liquid from D. Ellis and P.J.W. (unpublished data). b, Results from experiments with 4.5 wt% H₂O; open circle denotes same starting mixtures as in a but containing 4.5 wt% H₂O; other symbols are the same as in a. Because of the closely spaced liquid compositions only one two-liquid field is shown.

the silicate fraction in the mixture 1 experiment contains olivine (86% forsterite, Fo_{86}) and aluminous spinel, and has ~34 wt% SiO_2 and ~30 wt% Na_2O (CO_2 -free basis). SiO_2 and Na_2O values in the coexisting carbonate liquid are ~10 and 61 wt%, respectively. Olivine (Fo_{86}) and spinel are more abundant in the experiment with mixture 2 at 1,250 °C. Runs using mixtures 3 and 4 (both with $\text{Dol}/\text{Na}_2\text{CO}_3 = 1$) produced olivine, clinopyroxene, spinel and carbonated silicate liquids at 1,250 °C. Clinopyroxene dominates the crystalline assemblage in both runs and the resulting liquids are driven toward the $(\text{Na}_2\text{O} + \text{K}_2\text{O})-(\text{CaO} + \text{MgO} + \text{FeO}^*)$ join. Mixture 5 with $\text{Dol}/\text{Na}_2\text{CO}_3 = 3$ was largely crystalline at 1,250 °C, and the small pockets of liquid could not be analysed using SEM area scans.

The 1,150 °C tie line defines the extent of immiscibility in the mixture 1 + 6% apatite ($\text{Dol}/\text{Na}_2\text{CO}_3 = 0$). The silicate fraction contains olivine (Fo_{83}) and spinel. Although the two-liquid field has expanded slightly relative to the 1,250 °C boundary, this may simply reflect the 100 °C drop in temperature. Apatite does not seem to have a large effect on the size of the two-liquid field. For a variety of compositions, the silicate-carbonate immiscibility field extends from the $(\text{SiO}_2 + \text{Al}_2\text{O}_3 + \text{TiO}_2)-(\text{Na}_2\text{O} + \text{K}_2\text{O})$ join to close to the $\text{CaO} + \text{MgO} + \text{FeO}^*$ corner at crustal pressures (Fig. 1a). The decrease in the size of the anhydrous two-liquid field at 25 kbar compared to 5 kbar must reflect the increasing solubility of dolomite in silicate liquids with increasing pressure.

Figure 1b shows the results of the hydrous experiments. Runs using mixture 1 + 4.5% H_2O ($\text{Dol}/\text{Na}_2\text{CO}_3 = 0$) display immiscibility over the range of investigated temperatures (1,200–1,050 °C). At the lowest temperature the two liquids coexist with olivine. Experiments using mixture 2 + 4.5% H_2O ($\text{Dol}/\text{Na}_2\text{CO}_3 = 0.33$) contain a single liquid and minor olivine at 1,150 °C and a single liquid, olivine (Fo_{86}) and amphibole at 1,100 °C. Although we have not run experiments down to 950 °C, the solidus of fertile peridotite + vapour³, we do not expect the two-liquid field to expand significantly between 1,050 and 950 °C. Further, below, 1,100 °C increasing crystallization of olivine + amphibole ± clinopyroxene will drive residual liquids toward the $(\text{Na}_2\text{O} + \text{K}_2\text{O})-(\text{CaO} + \text{MgO} + \text{FeO}^*)$ join and out of the immiscibility field.

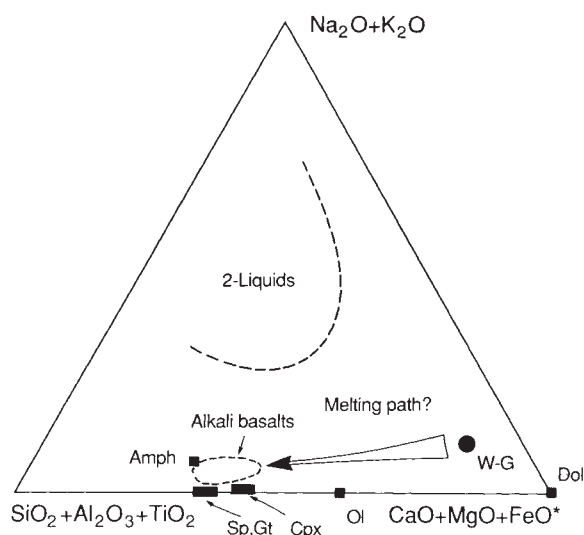


FIG. 2 Anhydrous 25-kbar two-liquid field and an inferred melting path for amphibole + dolomite peridotite projected onto the $(\text{SiO}_2 + \text{Al}_2\text{O}_3 + \text{TiO}_2)-(\text{CaO} + \text{MgO} + \text{FeO}^*)-(\text{Na}_2\text{O} + \text{K}_2\text{O})$ plane. W-G denotes the carbonate liquid composition (Table 1, column 3)³; alkali basalt field^{12,15}; squares and rectangles denote minerals that could crystallize from carbonate-rich liquids: olivine (Ol) and spinel (Sp) from this study, amphibole (Amph) from Wallace and Green³, garnet (Gt) and clinopyroxene (Cpx) from M.B.B. and P.J.W. (unpublished data).

Silica, titania, alumina and iron are enriched in the silicate relative to the carbonate liquid (Table 1). The behaviour of titanium and aluminium indicates that phases such as ilmenite, spinel and garnet will have relatively low solubilities in carbonate melts. Thus partial melting of dolomite-bearing peridotite may be an effective mechanism for fractionating high-field-strength elements from other incompatible elements and light from heavy rare-earth elements. Calcium, sodium and phosphorus are enriched in the carbonate relative to the silicate liquids. In the anhydrous experiments K_2O is partitioned into the carbonate liquid, whereas in the runs with water K_2O is enriched in the silicate liquid.

Figure 2 shows the anhydrous 1,250 °C two-liquid field projected onto the $(\text{SiO}_2 + \text{Al}_2\text{O}_3 + \text{TiO}_2)-(\text{CaO} + \text{MgO} + \text{FeO}^*)-(\text{Na}_2\text{O} + \text{K}_2\text{O})$ plane. The point labelled W-G is the carbonatitic melt produced by Wallace and Green³ at 1,000 °C and 22 kbar and in equilibrium with olivine, orthopyroxene, clinopyroxene, amphibole, spinel and ilmenite. The region labelled alkali basalts includes a variety of nepheline normative liquids with $\text{Mg}/(\text{Mg} + \text{Fe}^*)$ ratios > 0.66 (refs 12, 15). These basaltic compositions are generally assumed to represent partial melts from a garnet peridotite source^{16,17}. Figure 2 shows that a projected straight-line melting path from W-G to the alkali basaltic field does not intersect the two-liquid field. Note that the path is curved in CO_2 space because volatile contents decrease with increasing melt fraction; W-G contains ~45 wt% volatiles whereas most alkali basalts probably have < 5 wt% H_2O and CO_2 . There is no reason, however, to expect projected melting paths to curve toward the $\text{Na}_2\text{O} + \text{K}_2\text{O}$ corner. Thus it seems unlikely that immiscible silicate and carbonate liquids can be directly produced by vapour-undersaturated partial melting of hydrated dolomite peridotite at pressures of ~25 kbar. Also plotted on Fig. 2 are the compositions of olivine, clinopyroxene, amphibole, garnet, spinel and dolomite that could fractionate from carbonate-rich liquids. Residual liquids could only reach the two-liquid field after appreciable crystallization of dolomite and olivine.

Based on our vapour-undersaturated experiments we conclude that carbonatitic melts are unlikely to be produced by immiscible exsolution from carbonated silicate liquids at pressures between 20 and 30 kbar. The immiscibility field in both sets of experiments is far removed from natural mantle melts. We do not expect the presence of excess vapour to modify this conclusion. High-pressure partial melting and liquid immiscibility at lower pressures, however, remain viable processes for generating carbonatitic melts. We are now measuring how the immiscibility field expands with decreasing pressure. We anticipate that carbonated silicate melts derived at higher pressures may intersect a two-liquid field between 10 and 20 kbar⁵⁻⁷. □

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Macromolecular resolution of fossilized muscle tissue from an elopomorph fish

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THE Santana Formation (Lower Cretaceous, Aptian/Lower Albian) of Ceara, Brazil, contains many exceptionally preserved fish fossils within carbonate concretions^{1,2}. These concretions formed before compaction of the sediment and nucleated around carcasses of fish killed in mass-mortality events. Some concretions may contain several fish, preserved in three dimensions with fully articulated skeletons¹. A few specimens contain calcium phosphate in the form of cryptocrystalline hydroxyapatite. This occurs as coatings on bones and as a replacement of tissues. Samples of mineralized soft tissues can easily be liberated from the concretions by immersion in 10% acetic acid³. Of several mineral phases often preserving soft tissues, calcium phosphate and silica probably offer the greatest resolution of detail⁴. Fossilized soft tissues are most frequently found in fishes, although in the Santana Formation pterosaur wing membrane⁵ and the cuticles of arthropods^{6,7} have been reported. I report here the study of striated muscle tissue from a fossil elopomorph fish⁸ from this formation, in which subcellular ultrastructural features are distinguishable. The exceptional degree of preservation of the specimen raises interesting questions about the mechanism of fossilization.

A specimen of the elopomorph fish⁸ *Notelops brama* (Agassiz) in which phosphatization of soft tissues has been extensive, indicates that preservation occurred at the macromolecular level before and during postmortem degradation of the tissues⁹. This

resulted in the detailed replacement of structures at the sub-cellular level in some tissue types. This is most marked in phosphatized striated muscle tissue, where individual muscle fibres are preserved. The muscle fibres are replaced by phosphate in which individual crystallites are less than 100 nm long. Crystallites may be aggregated into microspheres with diameters of 1–2 μm , or as roughly cubic aggregates 0.7–0.8 μm in diameter. In the latter case, preserved muscle fibres show excellent detail of ultrastructure. Here crystallite orientation seems to have been controlled by the orientation of structural proteins, and other biomacromolecules within the fibres. The most obvious result of this is the retention of the sarcomeres in striated muscle fibres (Fig. 1a). This fossil banding, equivalent to the I and A bands of modern striated muscle, is highlighted by parallel rows of paired hydroxyapatite crystallite aggregates (Fig. 1b). The boundary between crystallites within crystallite pairs may correspond to the H band of individual sarcomeres, whereas the boundary between adjacent crystallite pairs may represent the Z band. Sarcomere length in the fossil material is $\sim 2 \mu\text{m}$ in compact fibres. Some fibres show that separation between lithified sarcomeres has occurred, but this is most likely a post-lithification artefact. The process of phosphatization took place during active decomposition of the muscle fibres, and individual fibrils can frequently be observed.

Other ultrastructural features of the muscle fibres which have been preserved include the sarcolemma (Fig. 1c) and rows of cell nuclei along the muscle fibre surfaces (Fig. 1d). In the former case there is sometimes no phosphatization of the enclosed muscle fibre, resulting in a stack of phosphatized sarcolemma membranes. The length of muscle fibres varies according to the size of the fish, and hence to the length of individual myoseptal blocks. Fibre diameter is generally of the order of 50 μm , and preserved sarcolemma is $\sim 1 \mu\text{m}$ thick. Cell nuclei have been observed on several muscle fibres. The nuclei are ovoid, $\sim 8 \mu\text{m}$ long and 4 μm in diameter, and arranged

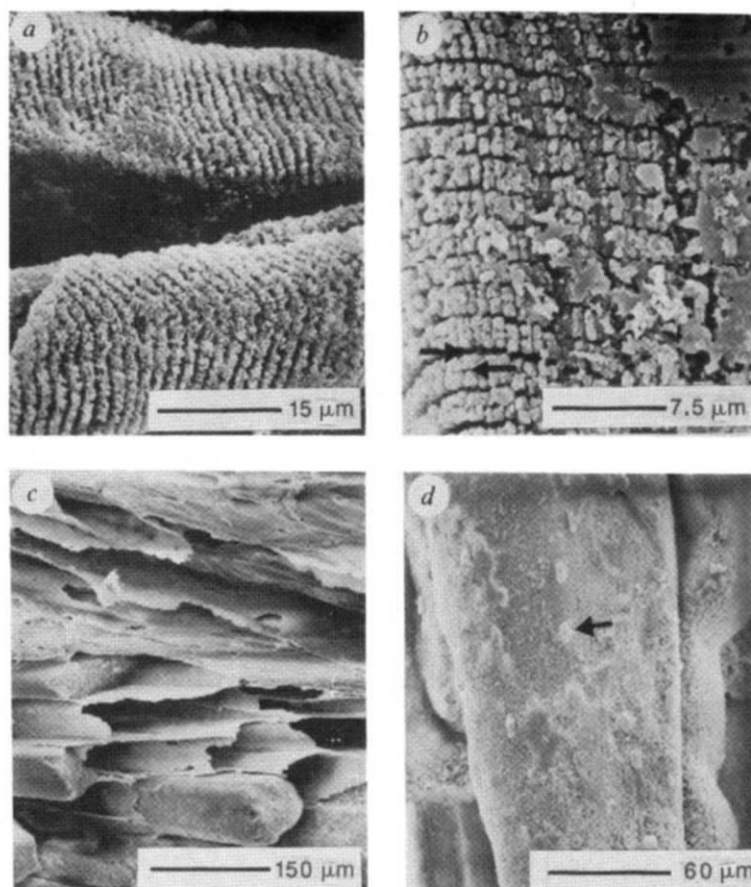


FIG. 1 Scanning electron micrographs of soft tissues from the Cretaceous actinopterygian fish *Notelops brama* (Agassiz), preserved by metasomatic replacement with calcium phosphate and prepared in 10% acetic acid. a, Two striated muscle fibres showing well-preserved banding. b, Detail of sarcomere preservation showing individual crystallite aggregates of calcium phosphate arranged in pairs; arrows indicate zones interpreted as H and Z bands. c, Unusual situation where preservation of sarcolemma has occurred, but preservation of fibres is incomplete. The result is a block of three-dimensionally preserved cell membranes. d, Striated muscle fibre showing patches of preserved sarcolemma, sarcomeres and rows of cell nuclei (arrowed).

in rows with long axes parallel to the length of the muscle fibre.

The presence of exceptionally preserved soft tissues raises several questions regarding both the rate and mechanism of fossilization. Many models for exceptional preservation require rapid burial, anoxicity, or both¹⁰⁻¹². There is evidence for scavenging of many of the exceptionally preserved Santana specimens suggesting that anoxic and rapid burial models are not applicable here. The Santana fossils are associated with laminated organic material, and I suggest that bacterial sealing as proposed by Seilacher *et al.*¹¹ is a more appropriate model for their exceptional preservation.

Bacterial breakdown of soft tissues might be inhibited by sterilization of bottom waters by high salinity or very low temperatures, thus prolonging their availability for the slow process of fossilization. At a palaeolatitude of 10° S, the Santana Basin was unlikely to have been flooded by very cold water, but bottom water of high salinity may have been present¹ as evaporites occur below.

For soft tissues to be so well preserved mineralization must either take place rapidly (in hours rather than days) or bacterial decomposition must be inhibited¹². Most models regard inhibition of bacterial decomposition as an acceptable mechanism; keeping the soft tissues 'on ice' while fossilization takes place. Gill and muscle degrade rapidly after death; within the first hour such tissues lose their rigidity and may display collapse structures. Santana fossil tissues show preservation of these features, suggesting that phosphatization occurred during active decomposition⁹.

The mechanism of phosphatization is not fully understood. It may in part be analogous to the biomineralization process in vertebrates. Here, ultramicroscopic crystallites of apatite nucleate within specific sites in collagen or collagen-like proteins¹³. This mechanism is a possibility for the fossil fish because of the abundance of tissues rich in structural proteins. But phosphatizing bacteria have been reported in sediments, where they have an important role in the production of phosphorites¹⁴. Shapiro¹⁵ demonstrated that environmental fluctuations (changes in temperature and/or oxygen levels) can induce rapid precipitation of phosphate by microorganisms, and a similar bacterially mediated process may be important in the fossilization of soft tissues^{1,16}. □

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Experimental reversal of courtship roles in an insect

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STUDIES of sexual selection¹ commonly examine female mating preferences or intermale competition², but there have been few experimental tests of factors controlling why these, the 'typical courtship roles', exist in the first place. Although the distribution of mates can affect sexual selection, and thus the degree of sexual competition³, it is the typically lower investment in offspring by males relative to females that is thought to determine the usually greater potential mating frequency, and hence greater sexual competition, among males^{4,5}. Reversal in the typical courtship roles is, therefore, expected to occur where there is high male parental investment⁴. The courtship roles are generally considered to be fixed for any particular species because of the usually inflexible nature of adaptations for parental care. Certain katydids (Orthoptera: Tettigoniidae)⁶⁻⁸, however, show intraspecific variation in these roles. This has been suggested to result from variation in the relative importance of male parental investment⁹ in the production of offspring. Male parental investment comprises the nutritional value of the spermatophore eaten by the female. When other food is scarce therefore, females are predicted to compete sexually for males and their offerings^{7,8,10}. Here we confirm this prediction and show that an increase in food from a low level results in a change from role-reversal to the typical roles; this supports the theory^{4,5} that variation in relative male parental investment controls sexual differences.

Both male and female katydids invest parentally in eggs. The male transfers a meal consisting of the spermatophylax (part of the spermatophore) to his mate, the nutrients of which are translocated to eggs¹¹ and increase both number and fitness of

offspring sired^{9,12,13}. For several species, the fitness-enhancing benefits to females of this costly¹³⁻¹⁵ nutrient gift have apparently led to sexual selection among females associated with both mate choice by males and inter-female aggression^{6-8,16}. In these species, however, this courtship role-reversal is found only in certain populations. Such a role-reversal is argued to be controlled by food availability⁶⁻⁸ because a scarcity of food: (1) decreases the number of sexually active males and thus their large spermatophylax contributions; and (2) increases the mating (spermatophylax-acquiring) frequency of hungry females¹⁵. Therefore, with such a decrease in food availability in nature, males rather than females are predicted to be the choosy sex because of the relative scarcity of males that have gained enough nutrients to donate parental investment. Because of the cost of the male contribution¹³⁻¹⁵ (ultimately in terms of future offspring sired^{5,9}), its enhanced value represents an increase in relative male parental investment^{7,10}. An experimental test of the hypothesis that diet controls sexual differences would, therefore, represent a test of the influence of relative parental investment.

The subject is an undescribed genus and species of zaprochiline katydid that feeds only on the pollen of certain spring-flowering plants^{17,18}. Large numbers of feeding individuals and sexually signalling (calling) males can be found on a single food plant¹⁸. In nature the species showed a courtship role-reversal early in the season when feeding from flowers, particularly kangaroo paws (*Anigozanthos manglesii*), that seemed to produce relatively little pollen. Later in the season, when there was an abundance of pollen available on the long flower spikes of *Xanthorrhoea preissii*, fewer females were mating and females became choosier than males⁸. Although this switch in mating system was correlated with an apparent increase in pollen availability, alternative causal explanations, such as a decrease in mating activity by older females, are plausible⁸.

In the present experiments, the effects of such variables were controlled by directly manipulating the amount of pollen available; during the flowering of kangaroo paws early in the season, four caged populations of katydids received supplemental food and four served as controls (Table 1).

TABLE 1 Differences between control and supplemental food treatments

Treatment	Cage	Males			Females			Sexual behaviour		
		Mean ($\bar{x}$) No. of calling males per sample ($n=11$)	Mean mass of spermatophore glands in mg (n)	Mean number of eggs in ovaries (n)	Mean weight of mature eggs per ♀ in mg (n)	Mean number of matings per female (n)	Opportunity for sexual selection on females ($s^2/\bar{x}$) (n)	Fraction of interactions with ♂ choice (n)	Fraction of interactions with ♀ choice (n)	Fraction of interactions with ♀-♀ competition (n)
Control	1	0.4	8.8 (19)	4.9 (23)	2.3 (6)	1.4 (24)	0.93 (24)	0.38 (13)	0.08 (12)	0.15 (13)
	3	0.7	8.8 (18)	5.5 (21)	2.2 (12)	1.2 (24)	0.94 (24)	0.44 (9)	0.11 (9)	0.22 (9)
	4	0.1	8.8 (20)	5.9 (22)	2.2 (10)	1.4 (24)	1.16 (24)	0.55 (11)	0 (11)	0.27 (11)
	6	0.3	10.2 (20)	5.7 (23)	2.2 (11)	1.3 (24)	1.06 (24)	0.31 (13)	0 (13)	0.30 (13)
Supplemental food	2	8.1	17.0 (21)	12.5 (24)	2.5 (22)	0.6 (25)	0.52 (24)	0 (8)	0.69 (8)	0 (8)
	5	4.7	13.6 (21)	5.7 (19)	2.4 (10)	0.7 (24)	0.55 (24)	0.14 (7)	0.43 (7)	0 (7)
	7	5.7	14.9 (22)	7.5 (20)	2.6 (10)	0.8 (24)	0.49 (24)	0 (5)	0.20 (5)	0 (5)
	8	7.9	18.5 (23)	11.0 (24)	2.5 (20)	0.6 (23)	0.86 (23)	0.06 (8)	0.38 (8)	0 (8)
Difference between treatments (Mann- Whitney test) (P)		0.002	0.002	0.08	0.002	0.002	0.002	0.002	0.002	0.002

The species is catalogued in the Australian National Insect Collection (ANIC) as Gen. Nov. 22, sp. 1 of the Zaprochilinae (D. C. F. Rentz, in preparation). (Voucher specimens were deposited in the ANIC¹⁸.) The study site, also that of previous studies^{8,18} is native bush of King's Park, Western Australia²³. We placed cuboidal fibreglass-screened cages, 0.75 m linear dimensions, over eight clumps of flowering kangaroo paws for 14 days in September 1989. Four cages were randomly selected as supplemental food replicates and into each were placed two artificial *X. preisei* flower spikes produced by coating the distal 15–20 cm of dried sections of flower stalks first with dilute honey, then pollen (bee-collected from Western Australian plants). The remaining four cages were controls receiving only the dried stalks. We placed individually marked zaprochilines, 24 of each sex, into each cage (such densities occur in nature). The behaviour of insects in one replicate of each treatment was observed each evening for a total of 12 sampling days. Observations on focal cages were made from sunset (~18:30) until ~22:00, the period of mating activity⁸. At about 20:30 each day (except one, lost due to rain), we censused the calling males and mated (spermatophore-carrying) females in each of the nonfocal cages. They seemed to behave naturally in the cages; as in nature^{8,18}, almost all calling males perched on the stems of food plants allowing the observer unobstructed views (using red light) of sexual interactions. At the end of the experiment all insects were collected and later measured and dissected to determine fecundity, weight of (two randomly selected) eggs, and spermatophore gland weight.

Mating in this species begins when a receptive female mounts a male after responding to his ultrasonic call¹⁸. After coupling the male bends behind the female and the spermatophore is transferred several minutes later. Following separation the female bends and starts to eat the gelatinous spermatophylax which surrounds the ampulla^{8,18} (the ampulla transfers sperm into the female). Mate rejection was recorded if the male or female moved away from its partner before spermatophore transfer⁸. Following rejection by a male, females often followed the male and recoupling sometimes occurred. In the case where a female rejected a male, males groped their terminalia in the direction of the retreating female. Only males can reject mates after pairs have coupled genitalia⁸. Sexual competition between females occurs when two females grapple either while responding to the same calling male or after a female disrupts a mating pair. No fighting between males has been noted in this species but it is possible that competition occurs through acoustical displays^{8,18}.

The results were as predicted. As for unmanipulated field populations⁸, there was a reversal in the courtship roles when the only food available to zaprochilines was pollen from kangaroo paws (control); such a reversal was not evident when the katydids received supplemental pollen. Quantitative results are presented in Table 1. To maintain statistical independence, if individuals were represented in more than one interaction only one (randomly determined) interaction was used. Fights between females over males were observed in control cages but never in food-supplemented cages. The frequency of rejection of females by males was significantly higher in the control cages, whereas the rejection of mates by females was most frequent in food-supplemented cages.

Courtship role-reversal in control cages was due to the predicted presence of fewer sexually active males than females; there were significantly fewer calling males in control cages than in food-supplemented cages. The greater proportion of sexually active males in supplemented cages was a result of the increase in the number of males obtaining sufficient pollen to produce spermatophylax donations. This is evidenced by the significantly larger spermatophore-producing glands of food-supplemented males relative to control males. After mating, male katydids call only when the spermatophore glands have been replenished¹³ (even when starved, such males do not reduce the size of the

subsequent spermatophore¹⁵). The difference between treatments in the ratio of sexually available females and males is due apparently both to the increase in spermatophore production by food-supplemented males and to the decrease in the mating (spermatophylax-acquiring) frequency of the now food-sated females in this treatment. The number of matings by females in supplemented cages was about half that observed in controls. This accords with observations of female zaprochilines on varying unmanipulated diets in the field⁸. Fecundity and egg size are increased both by additional spermatophylax¹³ and apparently by additional pollen⁸ eaten, so the higher mating frequency of control females probably acts to increase fecundity. Additional courtship meals in our experiments, however, did not seem completely to compensate for the decreased availability of pollen because control females had lower fecundities and smaller mature eggs compared to food-supplemented females (Table 1).

The opportunity for sexual selection to favour particular females was estimated in a between-treatment comparison of the distributions of variances in female mating success ($s^2/\bar{x}$) (refs 19, 20). As predicted, variances were significantly higher in control than supplemented cages. The body mass of females is expected to be a sexually selected trait as males of this⁸ and other katydids^{7,16} prefer heavier, more fecund females and larger female zaprochilines usually win fights over pollen⁸. Thus we predicted a mating advantage for heavy females in control but not in food-supplemented cages. This prediction was supported. The regression of body mass on mating frequency was significant for the control (slope = 0.09, $r^2 = 0.10$, $F_{1,85} = 9.6$, $P = 0.003$) but not for supplemented cages (slope = -0.01, $r^2 = 0.01$, $F_{1,78} = 0.9$, $P = 0.34$) (the four replicates within each treatment were pooled after showing that slopes within treatments were homogeneous ($P > 0.05$)). Such a relationship in low cages is, however, predicted not only by the mating success hypothesis but also by the fact that more courtship meals (matings) may increase female mass.

A decrease in pollen available to the zaprochilines causes male parental investment in the nutritious spermatophore to increase in value for offspring production. This change in value does not, however, result in an increase in female choice of males able to supply high quality donations because there is no opportunity for such choice as a result of the large reduction

in the proportion of nurturant males. This relative scarcity of males is caused not only by the fact that few males have obtained enough pollen to produce a spermatophore, but also by an increase in mating frequency of females as they seek additional courtship meals¹⁵. Thus females are subjected to heightened sexual selection associated both with fights between females for access to nurturant males and with mate choice by males. We conclude that the facultative nature of courtship roles in this species results from variation in the relative importance of male parental investment. The results refute alternative explanations for similar behavioural variation in nature^{6,8}, including the suggestion that such variation may be due to differences in population density²¹. The fact that manipulation of relative male parental investment¹⁰ controls sexual differences also argues against the contention that increased male parental investment results from decreased competition between males¹⁹, rather than the converse as argued by Williams⁴ and Trivers⁵.

Variation in relative parental investments thus seems to cause greatly differing patterns of sexual selection in brief spans of ecological time. Variation in resource availability is likely to determine the relative importance of the male contribution in any species in which females or offspring receive food from the mate. Such paternal investment occurs in a variety of taxa, and includes the donation of prey in birds, and of prey and a variety of male-produced secretions in insects²². Therefore, the facultative nature of the courtship roles is unlikely to be unique to spermatophore-consuming katyids. □

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Cortical microstimulation influences perceptual judgements of motion direction

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NEURONS in the visual cortex respond selectively to perceptually salient features of the visual scene, such as the direction and speed of moving objects, the orientation of local contours, or the colour or relative depth of a visual pattern. It is commonly assumed that the brain constructs its percept of the visual scene from information encoded in the selective responses of such neurons. We have now tested this hypothesis directly by measuring the effect on psychophysical performance of modifying the firing rates of physiologically characterized neurons. We required rhesus monkeys to report the direction of motion in a visual display while we electrically stimulated clusters of directionally selective neurons in the middle temporal visual area (MT, or V5), an extrastriate area that plays a prominent role in the analysis of visual motion information^{1–8}. Microstimulation biased the animals' judgements towards the direction of motion encoded by the stimulated neurons. This result indicates that physiological properties measured at the neuronal level can be causally related to a specific aspect of perceptual performance.

Like other cortical sensory areas, MT is organized in a columnar fashion so that clusters of neighbouring neurons have similar physiological properties⁹. In MT, neurons in a single cortical column discharge a burst of action potentials in response to motion in a 'preferred' direction, but yield little or no response to motion in the opposite or 'null' direction. The preferred direction of motion varies systematically from column to column so that MT contains a complete representation of motion direc-

tion at each location in the visual field. Consequently, a microstimulation current that selectively elevates the discharge rate of a small cluster of MT neurons should enhance the intracortical signal related to a particular direction of motion. In primate motor cortex, a 10- μ A pulse of cathodal current directly activates neurons within $\sim 85 \mu\text{m}$ of the electrode tip¹⁰. To activate local clusters of MT neurons, we therefore applied 10 μ A stimulating pulses (0.2-msec pulses, 200 Hz, biphasic) to selected sites in which neurons encountered over 150 μm of electrode travel had similar preferred directions. Although we attempted to confine direct excitation to a local cluster, neurons remote from the stimulation site were probably activated trans-synaptically¹¹. But activation of remote neurons does not necessarily imply a loss of functional selectivity; there is increasing evidence that cortical columns are preferentially connected with other columns having similar response properties^{12,13}. These considerations suggest that microstimulation in our experiments may have activated a circuit of neurons encoding a particular direction of motion.

Our methods for electrophysiological recording and for monitoring eye movements in trained rhesus monkeys were adapted from those of Wurtz and colleagues^{14,15}, and our psychophysical methods were based on those described by Newsome and Paré⁶. Figure 1 illustrates the procedures used in the present experiments. In brief, three rhesus monkeys were trained to discriminate the direction of motion in a random dot display shown on a video screen. In the display, a specifiable percentage of the dots carried a constant-velocity or 'correlated' motion signal while the remaining dots moved in random directions, creating a masking motion noise. We varied the strength of the motion signal by changing the percentage of dots in correlated motion. For each experiment a new stimulation site was selected and the motion display was placed in the multi-neuron receptive field mapped at the stimulation site. On a given trial the monkey maintained visual fixation on a stationary point of light while viewing the random dot stimulus for one second. The correlated motion signal was randomly selected to be in either the preferred or null direction of the local cluster of neurons. After the viewing interval, the monkey indicated its judgement of motion direction by making a saccadic eye move-

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ment to one of two light-emitting diodes corresponding to the possible directions of the motion stimulus. The monkey received a liquid reward for a correct choice. An experiment consisted of 640 randomly interleaved trials. On half of the trials we applied electrical stimulation that began and ended simultaneously with the onset and offset of the random dot stimulus. The monkeys performed trials at four correlation levels—0% correlation and three levels near psychophysical threshold. The reward contingencies were identical on stimulated and non-stimulated trials.

If microstimulation enhances the intracortical signal related to the preferred direction of motion of the local cluster of neurons, we would expect stimulation to bias the animal's psychophysical judgements towards that direction. Figure 2 shows the results of two experiments in which microstimulation had such an effect. For both experiments, the proportion of decisions in favour of the preferred direction ('preferred decisions') is plotted against the strength of the motion signal expressed as the percentage of dots in correlated motion. The closed symbols represent trials with electrical stimulation; open symbols correspond to nonstimulated trials. Positive correlations indicate motion in the preferred direction and negative correlations represent motion in the opposite direction. Comparing performance on stimulated and nonstimulated trials, one can see that at every correlation level the monkey made more preferred decisions when electrical stimulation accompanied the visual stimulus, with a net increase of 43 preferred decisions in Fig. 2a and 118 preferred decisions in Fig. 2b.

In both experiments of Fig. 2, the increase in preferred decisions due to microstimulation can be described as a leftward shift of the psychometric function. The magnitude of the leftward shift quantifies the microstimulation effect in units of the visual stimulus. In other words, the magnitude of the leftward shift, expressed as percentage of correlated dots, corresponds to the

visual stimulus change that would mimic the behavioural effect of microstimulation. We employed logistic regression analysis¹⁶ to measure the magnitude and statistical significance of the stimulation-induced shift of the psychometric function. For the experiment of Fig. 2a, the effect of microstimulation was behaviourally equivalent to the addition of 7.7% correlated dots to the visual stimulus and was highly significant ($P \leq 0.0001$). For the much larger effect in Fig. 2b, the effect of microstimulation was behaviourally equivalent to the addition of 20.1% correlated dots ($P \leq 0.0001$).

Microstimulation caused statistically significant shifts in the psychometric function ($P < 0.05$) in 18 of 38 experiments in one monkey, in 9 of 16 experiments in a second monkey, and in 3 of 8 in a third. Figure 3 shows for each experiment the magnitude of the stimulation-induced shift expressed as percentage of correlated dots. Positive values correspond to leftward shifts in the psychometric function and negative values correspond to rightward shifts. Striped bars indicate experiments in which the shift of the psychometric function was statistically significant. In 29 of the 30 experiments that yielded significant effects, microstimulation shifted the psychometric function leftwards, indicating an increase in preferred decisions. In the remaining experiment, microstimulation caused a highly significant rightward shift, an observation that could be explained if microstimulation had a large effect on nearby columns whose preferred direction was opposite to that of the target column. This is a plausible explanation for the single counterintuitive result as adjacent columns of MT neurons sometimes have opposite preferred directions⁹.

The data in Fig. 3 show that microstimulation biased the monkeys' perceptual decisions towards the preferred direction of the stimulated neurons. The result is consistent with the notion that focal microstimulation enhances the sensory representation of one direction of motion relative to others. An alternative

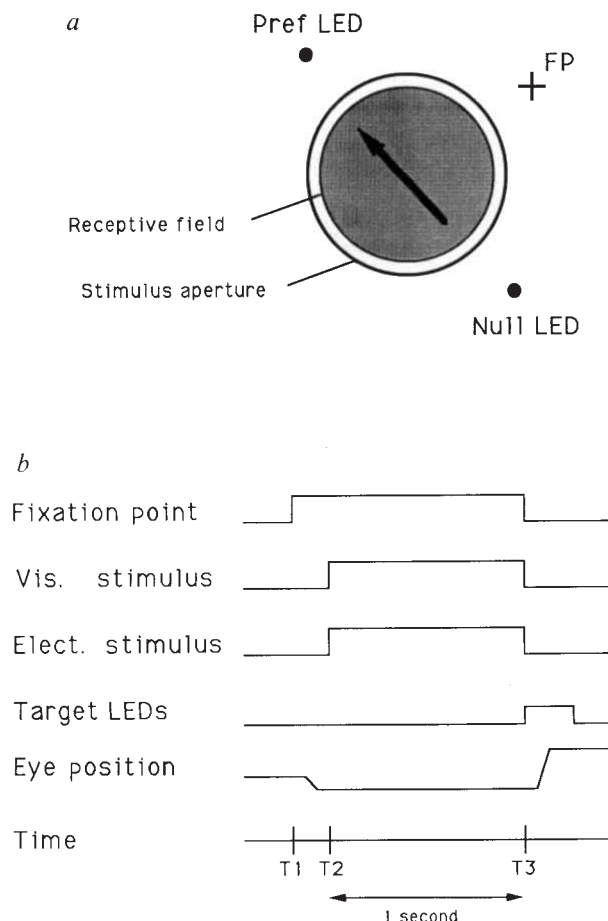


FIG. 1 The experimental protocol used to measure the effect of microstimulation on perceptual judgements of motion direction. *a*, Schematic diagram showing the spatial arrangement of the fixation point (FP), receptive field (shaded), stimulus aperture (thick circle) and response light emitting diodes (LEDs). While the monkey centred its gaze on the fixation point, we mapped the receptive field and identified the preferred direction (arrow) and speed of motion of neurons at the stimulation site. The random dot motion display was presented within a circular aperture whose dimensions and location were matched to those of the multi-neuron receptive field, and the speed of the motion signal was set equal to the preferred speed of the neurons. On a given trial, the motion signal in the visual display was randomly selected to be in the neurons' preferred direction (arrow) or in the null direction (opposite to arrow). The strength of the motion signal was randomly varied among four possible correlation levels: 0% correlation or one of three levels near psychophysical threshold. The monkey viewed the visual display for one second while maintaining its gaze on the fixation point. After the viewing interval, the monkey indicated its choice of motion direction by making a saccadic eye movement to one of two light emitting diodes (Pref LED and Null LED) that corresponded to the two possible directions of motion. Note that in all respects the visual display was tailored so that the demands of the psychophysical task were well matched to the information supplied by the neurons at the stimulation site. Since we tailored the visual display in this manner at each stimulation site, the spatial arrangement of the receptive field, stimulus aperture, preferred-null axis of motion, and response LEDs varied considerably from site to site. *b*, Schematic drawing illustrating the temporal sequence of events during a microstimulation trial. At time T1 the fixation point appeared and the monkey transferred its gaze to the fixation point, as indicated by the deflection in the eye position trace. At time T2 the visual (Vis.) stimulus appeared and the train of electrical (Elect.) stimulation pulses began. The monkey was required to maintain fixation for one second until time T3; if the monkey broke fixation during this interval the trial was aborted. The fixation point, visual stimulus and microstimulation pulses were turned off at time T3, and the target LEDs turned on. The monkey then indicated its judgement of motion direction by making a saccadic eye movement to one of the two response LEDs. A liquid reward was given for a correct choice, and an incorrect choice was penalized with a brief 'time-out' period. Unstimulated trials differed only in that no electrical stimulation accompanied the visual stimulus. The reward contingencies were identical for trials with and without stimulation.

explanation, however, is that microstimulation had a direct effect on the operant response, a saccadic eye movement. The latter hypothesis seems unlikely for several reasons. First, physiological recordings made during saccadic eye movements have failed to find any evidence of motor signals in MT¹⁷. Second, lesions of MT have no effect on saccades to stationary targets¹⁸. Third, control experiments show that microstimulation applied during the intertrial interval has no effect on eye movements. Finally, the long latency (>1 s) between the onset of microstimulation and execution of the saccade argues against a direct effect of stimulating current on motor signals. We therefore consider it highly probable that microstimulation affected the sensory signals underlying perceptual judgements of motion direction rather than motor signals related to the saccadic eye movement *per se*.

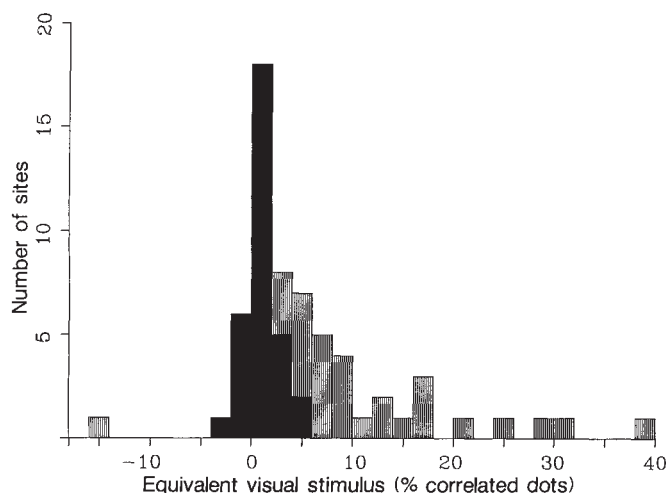


FIG. 2 Effect of microstimulation on psychophysical performance for two stimulation sites in MT. The abscissa indicates the strength of the motion signal in percentage of correlated dots. Positive correlation values indicate motion in the neurons' preferred direction; negative values correspond to motion in the opposite—'null'—direction. The ordinate shows the proportion of trials in which the monkey judged motion to be in the preferred direction of the stimulated neurons (preferred decision). Each data point is based on 40 trials, except for 0% correlation for which 80 trials were conducted. The correlation levels were selected so that the monkey would judge the direction of motion correctly in ~70% of the trials in a block. In half the trials (●) microstimulation was applied simultaneously with the visual stimulus; the other trials (○) contained no microstimulation. For each experiment we employed logistic regression analysis¹⁶ to fit sigmoidal curves of equal slope to the stimulated and nonstimulated data; the curves thus derived provided an acceptable fit to most of the data. *a*, Typical experiment in which stimulation biased the monkey's perceptual decisions towards the preferred direction of the stimulated neurons. The psychometric function for the stimulated trials was shifted leftwards by 7.7% correlated dots. The magnitude of the shift indicates that the behavioural effect of microstimulation could have been reproduced by adding to the visual stimulus 7.7% correlated dots in the preferred direction. *b*, Experiment in which microstimulation induced a leftward shift of 20.1% correlated dots, one of the larger effects we observed. The different appearance of the 'no stimulation' curves in *a* and *b* resulted largely from differences in choice bias in the two experiments. In the no stimulation condition in *a*, the monkey favoured the preferred direction on 56% of the trials at 0% correlation. Note that with no choice bias, the monkey would have made 50% preferred decisions at 0% correlation; in this experiment, therefore, the monkey had a very small choice bias. In the no stimulation condition in *b*, however, the monkey made preferred decisions on only 33% of the trials at 0% correlation. In this experiment, the monkey had a pronounced bias towards the null direction response LED; the effects of this bias are evident at other points on the no stimulation curve as well. Choice bias is a common phenomenon in human and animal psychophysics, and its presence and intensity in any particular experiment is difficult to predict. Most bias values observed in the present experiments were within the range observed in previous experiments that did not involve electrical microstimulation.

As the multi-unit receptive field at a given microstimulation site occupies a small portion of the visual field, it is desirable to know whether the perceptual effects of microstimulation are similarly localized in visual space. To answer this question, we required a monkey to perform the psychophysical task in the usual manner, but applied microstimulation to a topographically noncorresponding site in MT. In these experiments there was no overlap between the visual display aperture and the receptive field at the stimulation site. The effect of microstimulation was greatly attenuated or eliminated under these conditions. Thus the microstimulation effects were correlated with the spatial location, as well as the preferred direction, of the receptive field of the stimulated neurons.

Given the complexity of primate visual cortex, which contains more than 20 different visual areas with multiple anatomical interconnections, it is remarkable that local microstimulation of directionally selective neurons can cause a substantial change in psychophysical performance. This result may be less surprising if, as we suggested above, microstimulation trans-synaptically activates an extended circuit of neurons. This amplification of neuronal signals could be accomplished by activation of nearby columns in MT having a similar preferred direction and receptive field location, or by activation of similarly tuned neurons in visual areas other than MT. Although we do not know the full extent of cortex affected by microstimulation, the demonstrated correlation between neuronal preferred direction and psychophysical choice suggests that the activated neurons were functionally related to a particular direction of motion. The data therefore provide evidence causally relating neuronal activity to perceptually judged direction of motion. This experimental approach, which combines electrical microstimulation with multi-neuron physiological analysis and an appropriate perceptual task, may also prove useful for investigating cortical

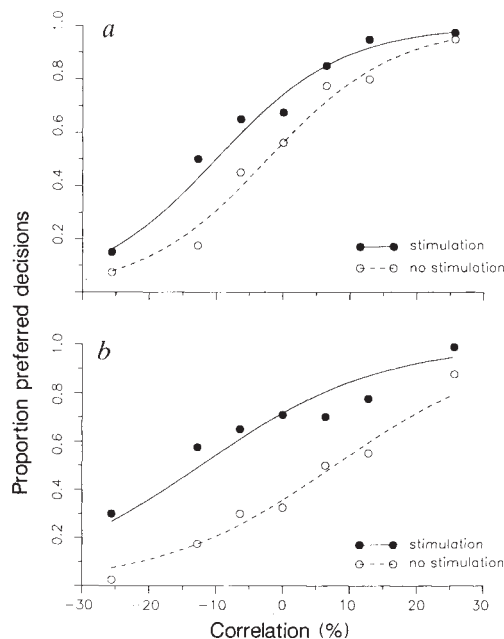


FIG. 3 Frequency histogram showing the magnitude of the stimulation-induced shift in the psychometric function for 62 experiments in three monkeys. The shift in the psychometric function may be thought of as an 'equivalent visual stimulus'—the visual stimulus change that would mimic the behavioural effect of microstimulation. Positive values indicate leftward shifts of the psychometric function; negative values indicate rightward shifts. The striped bars identify experiments in which the effect of microstimulation was statistically significant (logistic regression $P < 0.05$). In 29 of the 30 experiments with statistically significant effects, the stimulation-induced shift was leftwards, indicating an increase in decisions favouring the preferred direction of the stimulated neurons.

circuits that contribute to other aspects of visual perception such as orientation, colour and depth. □

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Presynaptic enhancement shown by whole-cell recordings of long-term potentiation in hippocampal slices

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LONG-TERM potentiation (LTP) of synaptic transmission in the hippocampus is a widely studied model system for understanding the cellular mechanisms of memory^{1–6}. In region CA1, LTP is triggered postsynaptically^{7–10} by Ca²⁺-dependent^{11,12} activation of protein kinases^{13,14}, but the locus of persistent modification remains controversial^{15–25}. Statistical analysis of synaptic variability has been proposed as a means of settling this debate^{3,26}, although a major obstacle has been the poor signal-to-noise ratio of conventional intracellular recordings. We have applied the whole-cell voltage clamp technique²⁷ to study synaptic transmission in conventional hippocampal slices (compare refs 28–30). Here we report that robust LTP can be recorded with much improved signal resolution and biochemical access to the postsynaptic cell. Prolonged dialysis of the postsynaptic cell blocks the triggering of LTP, with no effect on expression of LTP. The improved signal resolution unmasks a large trial-to-trial variability, reflecting the probabilistic nature of transmitter release^{31–35}. Changes in the synaptic variability, and a decrease in the proportion of synaptic failures during LTP, suggest that transmitter release is significantly enhanced.

We made whole-cell voltage clamp recordings^{27–30} from CA1 neurons in rat hippocampal slices (400–500 µm). Positive pressure was applied to the recording pipette during penetration of the slice; high-resistance (1–5 GΩ) seals on cell bodies 2–3 cell diameters below the surface were then obtained by suction. After breaking into the cell, synaptic currents were elicited with bipolar stimulation electrodes placed in the stratum radiatum of CA1. Stable synaptic recordings could be maintained for as long as 10 hours.

To optimize the chances of obtaining LTP under whole-cell recording conditions, we used internal solutions with minimal Ca²⁺ buffering to avoid block of LTP^{11,12}, and with Cs⁺ as the main cation to enhance voltage control of the postsynaptic membrane. We also included ATP and GTP in the internal solution to allow activity of protein kinases or GTP-binding proteins. After a stable baseline period of 10–15 min, we were able to induce LTP by pairing^{7,9,10} a steady postsynaptic depolarization to ~0 mV with continued activation of the test pathway (40 stimuli at 2 Hz). This pairing procedure resulted in LTP in 14 out of 18 experiments (Figs 1c, 3a and 4c). By contrast, no potentiation was found in transmission through a simultaneously monitored control pathway that did not receive presynaptic stimuli during the postsynaptic depolarization (Figs 1d, 3b). Furthermore, synaptic stimulation at 2 Hz without postsynaptic depolarization did not give synaptic enhancement ($n = 5$). Thus, the potentiation was synapse-specific and required a combination of presynaptic activity and postsynaptic depolarization, just as seen in conventional recordings of LTP^{1–6,36}.

To investigate whether diffusible cytoplasmic factors are involved in potentiation, we attempted to trigger LTP at different times after gaining whole-cell access (Fig. 1c, d). Pairing soon after starting whole-cell recording (~20 min or less) consistently resulted in LTP lasting >1 hour (Figs 1, 3 and 4). But no potentiation was found with pairing >30 min after whole-cell access (6 out of 7 experiments). One possibility is that some diffusible postsynaptic substance is needed to trigger LTP, but not to maintain the potentiation.

The low background noise of whole-cell recordings allowed us to study small responses evoked with minimal stimulation, only slightly stronger than the highest stimulus that gave all failures. With minimal stimulation, activation is thought to be restricted to a single synapse onto the monitored neuron^{25,37}. The resulting synaptic currents showed a large variability between trials that was much greater than the background noise, but their individual time courses were similar (Fig. 1a, b), supporting the view that they originated from the same synapse^{25,37}. There were occasional clear failures (Figs 1a and 4a, b), and sporadic spontaneous events that resembled the elicited response, ranging in amplitude from <1 pA to ~10 pA (Fig. 1b, inset).

To understand the basis of the synaptic variability and its possible relation to fluctuations in transmitter release, we modified presynaptic or postsynaptic functions by changing the bathing medium (Fig. 2). Raising the extracellular calcium concentration ([Ca²⁺]_o) to increase presynaptic release³⁸ enhanced the synaptic currents (Fig. 2a), and changed their amplitude histogram from a highly skewed distribution (Fig. 2b) to a nearly symmetrical bell-shape (Fig. 2c). By contrast, when we added low concentrations of the glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione³⁹ (CNQX) to modify postsynaptic responsiveness, the average synaptic current was dramatically reduced, but the shape of the distribution remained unchanged (Fig. 2a, e). The distributions before and after CNQX therefore matched closely when normalized by their means (Fig. 2d). We obtained similar results with CNQX at lower [Ca²⁺]_o (data not shown).

To derive a simple and revealing index of synaptic variability, we computed $V^{-2} = M^2/\sigma^2$, where V is the coefficient of variation, M is the mean synaptic current and σ^2 is the variance about M , for a given epoch of consecutive responses from t to $t + \tau$. We can compute the expected behaviour of M^2/σ^2 if we make assumptions about the mechanisms underlying synaptic transmission. In the simplified case of a binomial distribution of transmitter release, $M^2/\sigma^2 = (Npvz)^2/Np(1-p)(vz)^2 = Np/(1-p)$, where p is the probability of release for each of N available quanta, v is the vesicular content, and z is the postsynaptic response to a fixed amount of transmitter. Thus, M^2/σ^2 is independent of changes in z and is a useful measure of some changes in presynaptic function, but not all factors (for example,

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v). It increases in proportion to N , and at least linearly with p , as experimentally confirmed at the neuromuscular junction³¹. The lack of dependence of M^2/σ^2 on the postsynaptic responsiveness (z) holds for more general cases as long as z does not vary from trial to trial within a given epoch (see Fig. 2 legend).

Assuming z is constant for an epoch, the theory predicts that changes in M^2/σ^2 between epochs will result from changes in release characteristics but not postsynaptic modifications. This was tested by examining changes in M^2/σ^2 following interventions known to affect presynaptic or postsynaptic mechanisms. As Fig. 2*e* illustrates, raising extracellular calcium dramatically increased M^2/σ^2 . This was found for all experimental manoeuvres expected to affect presynaptic release: lowering magnesium, application of 4-aminopyridine (data not shown) or increasing the stimulus strength to recruit more afferent fibres (Fig. 2*f*; $n = 10$). In general, M^2/σ^2 increased at least as much as the mean synaptic current. By contrast, addition of CNQX produced no significant change in M^2/σ^2 , despite its large effect on synaptic transmission ($n = 5$; see Fig. 2*e, g*).

Does M^2/σ^2 change with LTP? Figure 3*b* compares M^2/σ^2 of the test pathway with that of the control (unpaired) pathway, for the 14 experiments showing potentiation of mean synaptic current (Fig. 3*a*). Following pairing, M^2/σ^2 increased significantly in the test pathway, with no change in the control

pathway. The increase in M^2/σ^2 with LTP could arise from various presynaptic mechanisms: an increase in the number of available vesicles; an increase in the probability of release of some or all vesicles; a non-uniform increase in the amount of transmitter in the vesicles; or changes in non-vesicular release. It would not be expected from uniform changes in the number, sensitivity or conductance of glutamate receptor channels, or in the effectiveness of charge transfer from spines to dendrites.

If the expression of LTP involves an increased probability of release or a greater number of available quanta, one would expect fewer failures of transmission during LTP. Figure 4 illustrates an experiment where failures and responses were clearly resolvable. The amplitude distribution histogram had a distinct peak at zero amplitude which matched the amplitude distribution of the noise (inset). The area under the 0 pA peak was used to estimate the percentage of failures. In this experiment, the proportion of failures decreased from 27% in control, to 11% during LTP. This decrease was a consistent finding in each of five experiments where failures could be clearly resolved ($n = 3$ with minimal stimulation and $n = 2$ with intracellular microelectrode stimulation of a CA3 neuron). The collected data from all five experiments showed a mean ($\pm$ s.e.m.) decrease in failures from $63 \pm 11\%$ in control, to $17 \pm 8\%$ after pairing, consistent with a greater likelihood of transmitter release during

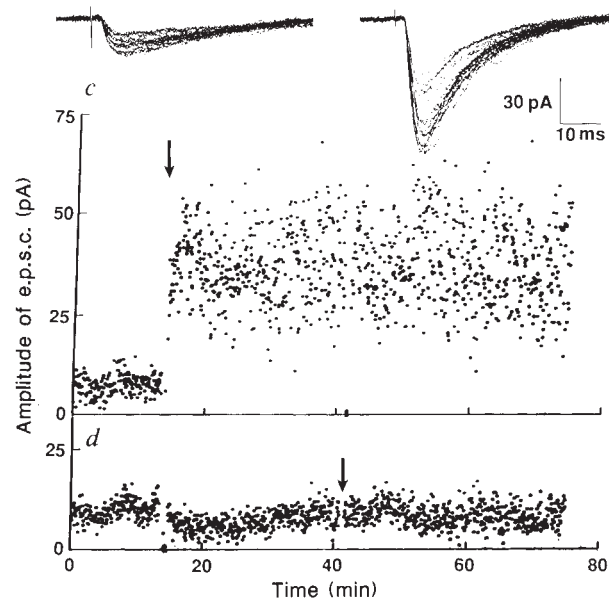
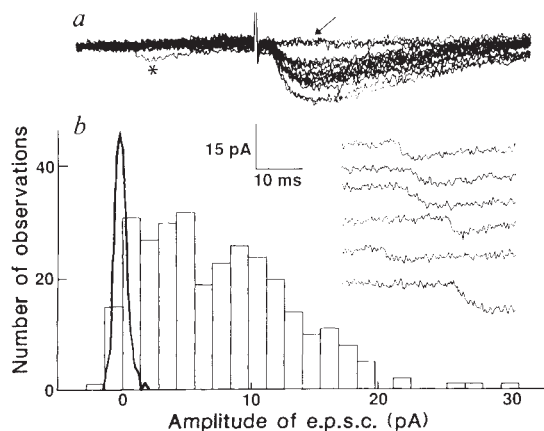


FIG. 1 Whole-cell recordings of synaptic transmission in conventional hippocampal slices show large inter-trial variability, synapse-specific LTP, and loss of induction but not expression, with prolonged dialysis. *a*, Sixteen consecutive records of excitatory postsynaptic currents (e.p.s.c.s), superimposed. Note spontaneous event in baseline period (asterisk) and synaptic failures (arrow). *b*, Amplitude distribution histograms for 200 consecutive synaptic currents (bars) and baseline noise (smooth curve). Bin sizes were 1.4 pA for e.p.s.c.s, and 0.14 pA for baseline noise. Inset: Representative spontaneous synaptic currents. Note the variability in amplitude and time course but an otherwise general similarity to evoked responses. *c, d*, Plots of synaptic responses against time in a postsynaptic cell receiving two independent inputs. *c*, LTP was selectively induced in one pathway by a pairing procedure 15 min after break-in (arrow); in the other pathway (*d*), the pairing procedure was applied 25 min later, but failed to induce LTP, in contrast to the maintenance of LTP in *c*. In the pairing procedure, the cell was depolarized from -70 mV to ~ 0 mV, while the paired (conditioned) pathway was stimulated 40 times at 2 Hz with no change in stimulus strength. Non-conditioned pathway received no stimuli during the postsynaptic depolarization. Inset: Families of consecutive current records showing e.p.s.c.s of conditioned pathway, collected before (left) and 30 min after pairing (right).

METHODS. Transverse hippocampal slices (400–500 μ m), obtained from 3–5-week-old rats by standard methods⁴², were submerged and superfused continuously with a modified Earle's solution containing (in mM): NaCl, 119; KCl, 2.5; $MgCl_2$, 1.3; $CaCl_2$, 2.5; NaH_2PO_4 , 1.0; $NaHCO_3$, 26.2; glucose, 11; picrotoxin, 0.02, and gassed with 95% O_2 /5% CO_2 (pH 7.4) at room temperature (22 $^{\circ}C$). A cut was made between regions CA3 and CA1 to prevent epileptiform activity. Synaptic transmission was elicited with bipolar stain-

less steel stimulating electrodes at two locations; each electrode delivered a stimulus every 4 s; stimuli from the two electrodes were separated by 2 s. E.p.s.c.s were recorded with a patch electrode (3–7 M Ω tip resistance, no fire polishing or Sylgard coating) in the whole-cell mode (Axopatch 1D). Pipette solution contained (in mM): Caesium gluconate, 100; EGTA, 0.6; $MgCl_2$, 5; ATP, 2; GTP, 0.3; HEPES, 40 (pH 7.2 with CsOH). Holding potential was kept constant at a level between -60 and -70 mV. E.p.s.c.s were amplified 50–500 fold, filtered at 1 kHz, digitized at 10 kHz and stored. For a given experiment, e.p.s.c. amplitudes were determined by averaging the current over a 10–20 ms window at the peak response and subtracting a baseline estimate from the same record. A similar procedure was used to measure the background noise before the synaptic stimulation³⁴. In a representative series of 17 experiments, the average synaptic current for minimal stimulation was 6.7 pA, in reasonable agreement with estimates of synaptic current from minimal e.p.s.ps recorded with high-resistance microelectrodes^{25,34}, after allowing for input resistance. Note, however, that the background noise in whole-cell recordings was considerably lower than in the most favourable microelectrode recordings^{25,34}. The input resistance in whole-cell recordings was typically 100–200 M Ω .

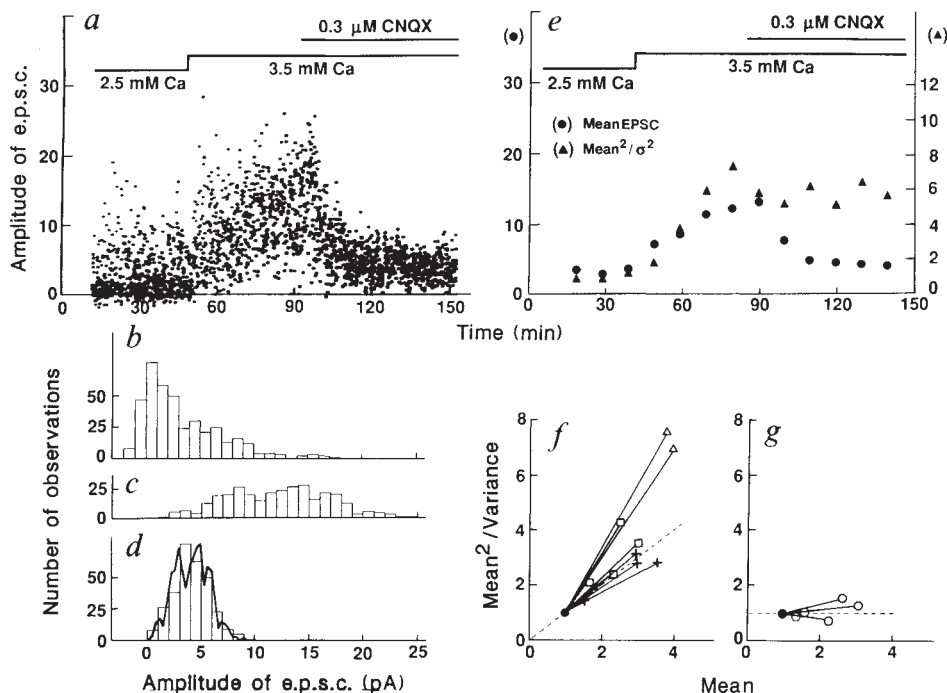
LTP. This conclusion is supported by the overall amplitude distribution, which changes from a skewed shape in control (Fig. 4*d*) to a bell-shape during LTP (Fig. 4*e*), as with increasing extracellular calcium (Fig. 2*b, c*).

One of our main findings is that whole-cell recordings can be used to study synaptic transmission over many hours in neurons

that are well below the surface of conventional brain slices (see also ref. 29). This approach is an alternative to methods that require cleaning of the surface of slices³⁰, and it allows access to deeper cells that are more likely to retain intact dendritic structures and functional properties.

Our experiments show that synapses can display LTP even

FIG. 2 Agents acting through presynaptic mechanisms affect shape of distribution histograms and M^2/σ^2 , whereas inhibition of postsynaptic receptors does not. *a*, E.p.s.c. amplitude plotted against time. Bathing calcium was raised from 2.5 mM to 3.5 mM, and 0.3 μ M CNQX was applied where shown. *b-d*, Amplitude distribution histograms for 300 e.p.s.c.s before manipulation (*b*), after elevation of calcium (*c*), and after subsequent addition of CNQX (*d*). Continuous line in *d* shows data from *c*, normalized to mean e.p.s.c. amplitude in *d*. Note close agreement of normalized histograms. *e*, Plot of mean e.p.s.c. amplitude, M (circles) and M^2/σ^2 (triangles) for the experiment shown in *a*, with $\tau=10$ min (150 trials). *f*, Plot of M^2/σ^2 against M for several presynaptic manipulations: concentration of calcium was increased from 2.5 to 3.5 mM (triangles); magnesium was decreased from 1.3 to 0.65 mM in the presence of 50 μ M 5-aminophosphonovaleric acid (squares); increased stimulus strength to recruit more fibres (cross). *g*, Plot of M^2/σ^2 against M for experiments in which CNQX (0.2–0.3 μ M) was applied to the bath. For each experiment in *f* and *g*, lines connect points obtained before and after manipulation; M and M^2/σ^2 were computed for 300 trials before the manipulation and for 300 trials at the peak of the effect, and normalized by the values corresponding to the smaller M for that experiment. Note that presynaptic manipulations thought to increase p affect M^2/σ^2 more than the mean, whereas increasing stimulus strength, which increases N , affects M^2/σ^2 as much as M . These findings are expected for a binomial release process (see text). Postsynaptic receptor blockade generally had no effect on M^2/σ^2 . This conforms with predictions of a treatment of synaptic responses that does not presuppose binomial statistics, uniformly sized packets, or even vesicular release. In the general case, the release process is considered to behave as a probability density function $R(x)$, where x is allowed to vary over possible values of transmitter release. The postsynaptic response is assumed to be zx , where z is a



constant postsynaptic response factor. From the elementary probability theory, the mean synaptic response is given by $\langle zx \rangle$ and the variance about the mean is $\langle (zx - \langle zx \rangle)^2 \rangle$, where $\langle \rangle$ denotes the expectation for trials within the epoch τ . Then $M^2/\sigma^2 = \langle zx \rangle^2 / (\langle (zx - \langle zx \rangle)^2 \rangle) = z^2 \langle x \rangle^2 / (\langle (zx)^2 \rangle - \langle zx \rangle^2) = z^2 \langle x \rangle^2 / (z^2 (\langle x^2 \rangle - \langle x \rangle^2)) = \langle x \rangle^2 / (\langle x^2 \rangle - \langle x \rangle^2)$. Thus M^2/σ^2 depends only on characteristics of the release process for that epoch, and not on z . This formalism assumes that postsynaptic responsivity is linear and does not vary from trial to trial (that is, z is constant) within the epoch τ , as found at the neuromuscular junction^{31,32}. It ignores the possibility of rapid and concerted changes in the receptivity of ensembles of receptors or a spatial heterogeneity in receptivity that responds differentially to independent sites of transmitter release.

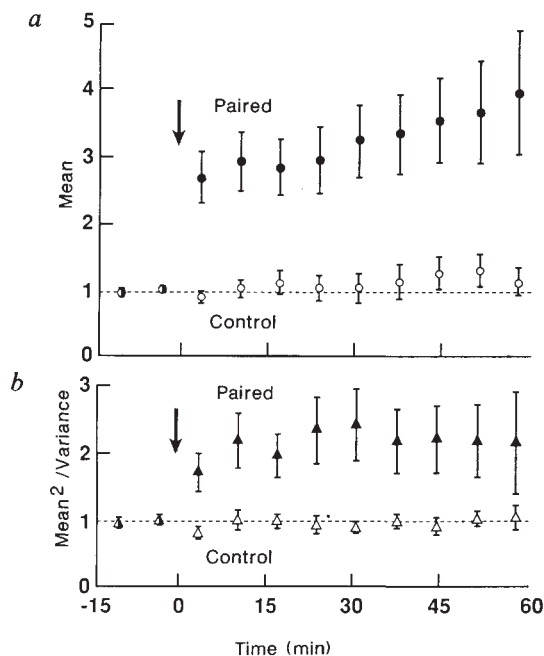


FIG. 3 A synapse-specific increase in M^2/σ^2 associated with LTP. Results from the 14 of 18 experiments that showed LTP with pairing (in the other four experiments with no LTP, M^2/σ^2 remained unchanged; data not shown). *a*, Ensemble averages and s.e.m.s of M for the paired pathway (filled circles) and the control pathway (open circles, $n=10$; in 4 experiments this pathway was not monitored). Arrow marks time of pairing procedure (as in Figs 1, 2). *b*, Ensemble averages of M^2/σ^2 for paired (filled triangles) and non-paired (open triangles) pathways for the same experiments as in *a*. Note that M increased more than M^2/σ^2 , particularly 30–60 min after pairing; the late change in M may reflect a delayed increase in postsynaptic responsivity²⁴.

after the postsynaptic cell has been accessed by a whole-cell recording pipette. This opens up the possibility of probing the postsynaptic mechanisms of LTP with a wide range of biochemical compounds, such as large proteins or nucleic acids, that could not be reliably delivered with intracellular microelectrodes. It is interesting that the ability to undergo LTP was lost after longer periods of whole-cell recording, as if some key cytoplasmic constituent were washed out, even though LTP established earlier during the recording did not decay. This contrast supports the idea that induction and persistence of LTP involve different molecular events, possibly in different locations^{13,40,41}.

Another advantage of whole-cell recordings is that they give much better signal-to-noise than conventional intracellular recordings, facilitating statistical analysis of small synaptic signals. Experiments conform with theory in supporting M^2/σ^2 as

a simple measure of changes in presynaptic function. In principle, this approach could be useful in characterizing neuroactive drugs whose locus of action is unknown.

Our results provide electrophysiological evidence for changes in presynaptic function during LTP, as already suggested^{13,15-19}. This conclusion is based on two independent but complementary approaches. The analysis of M^2/σ^2 is relatively model-independent, and does not require failures. M^2/σ^2 increased significantly and remained elevated during LTP; it was unchanged in pathways not undergoing potentiation (Fig. 3b). An analysis based on failures gives a more direct view of presynaptic function, but requires unitary events that are large relative to the background noise. In all cases where failures were clearly resolved, their occurrence was greatly diminished during LTP (see Fig. 4e for example). Thus, both sets of results support an increase in the likelihood of transmitter release.

Our results do not exclude some change in postsynaptic responsivity (Fig. 3 legend). This possibility²⁰⁻²² is supported by a recent report of minimal excitatory postsynaptic potentials (e.p.s.ps) in which no clear change in quantal content was detected in association with a 30% potentiation²⁵. The enhancement of transmitter release may have been more pronounced in our experiments because the potentiation was considerably larger (~200%).

Our evidence supporting presynaptic changes with LTP is striking in light of previous studies showing that induction is postsynaptic⁷⁻¹³. The combination of results makes it difficult to escape the conclusion that a retrograde message must travel from the conditioned postsynaptic cell to modify presynaptic function¹⁻⁵. As the proportion of failures decreases and M^2/σ^2 increases soon after pairing, the retrograde communication must occur promptly. □

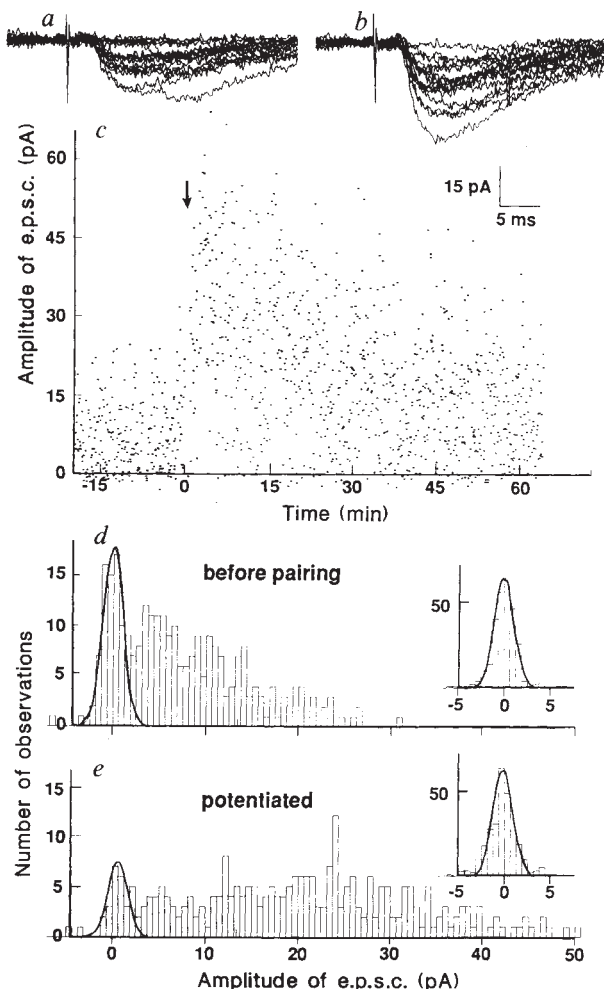


FIG. 4 Decreases in the proportion of synaptic failures associated with LTP. **a, b**, Groups of 16 consecutive e.p.s.cs taken before (**a**) and 40 min after (**b**) a pairing procedure to induce LTP. **c**, Amplitude of e.p.s.cs elicited by minimal stimulation plotted against time. Arrow marks pairing procedure (see Fig. 1). **d, e**, Amplitude distribution histograms for 17 min epochs before pairing (**d**, from $t = -17$ to $t = 0$) and after pairing (**e**, from $t = 5$ to $t = 22$ min). Smooth curves are Gaussians whose height was adjusted to fit the first peak in the amplitude distribution. The half-width of the Gaussians, 2.5 pA, was obtained by fitting amplitude histograms of the baseline noise during the same epochs (insets). The proportion of failures, estimated as the area under the Gaussian curve, was 27% before pairing (**d**), and 11% between 5 and 22 min after pairing (**e**). Note the change in shape of amplitude distribution histogram in association with 2.8-fold increase in mean synaptic current from **d** to **e**. During a later epoch (from $t = 40$ to $t = 57$ min, histogram not shown), the synaptic enhancement was 2.1-fold relative to control, and the proportion of failures was 15%.

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Role of abortive retroviral infection of neurons in spongiform CNS degeneration

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RETROVIRUSES are involved in several human neurological diseases with varying pathological features^{1–3}. Whether these diseases are due to a direct effect of the virus on nervous system cells is unknown. To gain insight into the pathogenesis of one retroviral neurological disease, we are studying the murine neurotropic retrovirus, Cas-Br-E, which causes lower motor neuron disease associated with spongiform degenerative changes in brain and spinal cord⁴. Central nervous system (CNS) injury seems to be due to direct viral action⁵, but the precise target cells of the virus are uncertain. After blood-borne virus enters the CNS it is found in capillary endothelial cells^{5–7}. No microscopic evidence for virus within glia or neurons has been found in some studies⁶, whereas virus or incomplete particles have been observed in CNS cells in other studies^{5,8}. Here we identify the neuron as a major target for Cas-Br-E in the CNS, suggesting that this disease may be a direct

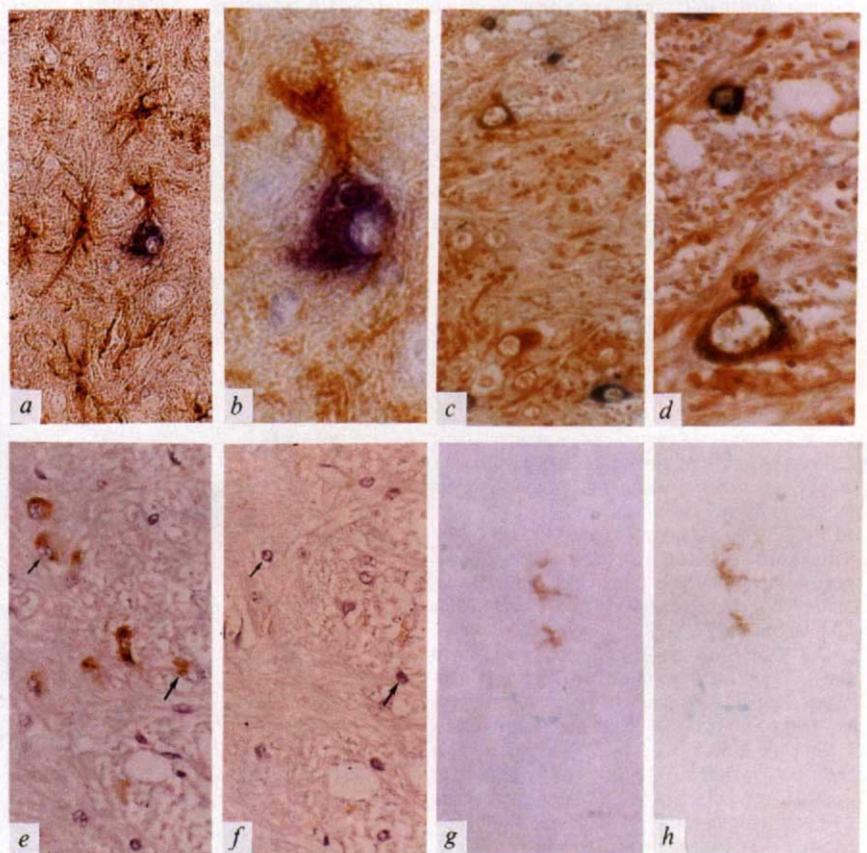
result of viral infection of neurons. We also show that envelope protein (Env, encoded by the *env* gene), a major determinant of neurovirulence^{9,10}, cannot be detected in neurons but is present in non-neuronal cells, although spliced *env* messenger RNA is synthesized in CNS tissue. This suggests that a post-transcriptional step in Cas-Br-E Env protein synthesis is impaired and that the neurological disease may be a consequence of abortive replication of virus in neurons. This may explain the failure to find neuronal infection in other neurological diseases by conventional methods of virus detection.

To determine the target cells for Cas-Br-E in the CNS, we compared the virus-host cell interactions of neurovirulent Cas-Br-E with that of non-neurovirulent Moloney murine leukaemia virus (MoMuLV). Whereas postnatal infection of mice with MoMuLV results in viral infection of primarily haematopoietic cells, mid-gestation infection of embryos allows MoMuLV to infect a wide variety of cells¹¹, including most cell types in the nervous system. Mice expressing even large amounts of virus in the nervous system do not, however, develop neurological disease. Mid-gestation infection with Cas-Br-E produces a disease that is clinically and pathologically the same as neonatal infection, except that its course is markedly shortened¹².

We infected mice intraperitoneally (i.p.) at birth or at mid-gestation. Spongiform degeneration and marked gliosis were observed in the spinal cord, brain stem, cerebellum and subcortical regions, as seen previously⁴. Cells identified morphologically as large motor neurons were stained when spinal cord sections of mice infected with Cas-Br-E i.p. at birth or at mid-gestation were treated with an anti-MoMuLV antibody that detected Cas-Br-E and MoMuLV viral antigens (Fig. 1). Other cells not readily identified under the light microscope also

FIG. 1 *a–d*, Double immunohistochemical staining of infected spinal cord sections. Note the colocalization of viral antigens and neurofilament antigens to the same cells. Brown, immunoperoxidase (IMP) staining of glial cells using antibodies to GFAP (*a, b*) or neurons using antibodies to neurofilaments (*c, d*); purple, alkaline phosphatase (AP) staining of virus-infected cells. *e–h*, Cells visualized by the anti-total virus antiserum were not stained with a polyclonal antibody recognizing gp70 in adjacent sections of Cas-Br-E infected spinal cord. Note lack of reactivity of anti-Env antibody (*f*) as compared with anti-total virus antibody (*e*). Arrows identify identical cells in the adjacent sections. These two antisera gave similar results in MoMuLV-infected tissues (*g*, anti-total virus; *h*, anti-Env). Brown, immunoperoxidase staining of virus-infected cells. Magnification: $\times 560$, *a, c, e–h*; $\times 1,400$, *b, d*. Counterstain in (*e*) and (*f*) is haematoxylin and in *g* and *h* is methyl green.

METHODS. SWR/J mice were infected i.p. as neonates with 0.05 ml virus with a titre of 10^5 XC plaque-forming units per ml or at mid-gestation⁷ with approximately 0.05 μ l virus of the same titre. Virus was prepared from cells producing cloned MoMuLV or Cas-Br-E (clone NE-8)¹⁷. Mice infected i.p. with Cas-Br-E were examined from 2 weeks to 5 months post-infection. Mice infected with Cas-Br-E or MoMuLV as mid-gestation embryos were examined at embryonic day 16, postnatal days 14 and 24 and at 2 and 3 months after birth (MoMuLV-infected mice). Four mice were examined at each time point. Mice were anaesthetized with sodium pentobarbital and perfused through the heart, first with 0.9% NaCl containing 100 units heparin per ml (Sigma) and then with Carnoy's fixative, both pre-chilled to 4 °C. Small portions of tissue were then post-fixed in Carnoy's at 4 °C overnight, dehydrated in graded alcohols and xylenes and embedded in paraplast plus. Serial 3- μ m sections were cut and heat-baked onto glass slides pretreated with Elmer's glue (Borden). Immunohistochemistry was performed as described¹⁸. For recognition of viral antigens, slides were incubated with goat antibodies to total virus (NIH 81S107) or to the viral envelope (gift of W. Schäfer) followed by a donkey anti-goat AP conjugate (1:100, Jackson Immunoresearch). Antibody localization was visualized by incubating sections with 1 mg ml⁻¹ Fast Blue BB (Sigma), 0.2 mg ml⁻¹ naphthol AS-MX phosphate in 0.1 M Tris buffer, pH 8.2. For detection of GFAP, sections were incubated with rabbit anti-GFAP antibody (1:800, Accurate), followed by a donkey anti-rabbit IMP conjugate (1:100, Jackson Immunoresearch). For detection of neurofilaments, the avidin-biotin system



used mouse anti-neurofilament antibodies (SM32, 1:4000, Sternberger-Meyer) followed by a mouse Vectastain ABC kit (Vector). Antibody localization was visualized using a peroxidase reaction with 3,3'-diaminobenzidine tetrahydrochloride (Aldrich) as the chromagen (1.5 mg ml⁻¹, with 0.1 ml of 3% hydrogen peroxide). Slides were counter-stained with methyl green or haematoxylin.

TABLE 1 Cas-Br-E and MoMuLV interactions with cells in the CNS

	Cas-Br-E		MoMuLV	
	Disease	Target cells	Disease	Target cells
Mid-gestation	Paralysis (15–30 days)	Neuron (endothelial, rarely astrocytes)	Leukaemia (3–5 months)	Neuron, glia endothelial
Postnatal	Paralysis (4–6 months)	Neuron (endothelial, rarely astrocytes)	Leukaemia (5–10 months)	None

contained viral antigens. Using antisera against glial fibrillary antigen protein (GFAP) to identify astrocytes, and antisera against neurofilament (NF) subunits to identify neurons, double immunohistochemical staining studies revealed that the vast majority of infected cells were neurons (Fig. 1*a–d*). Infection of astrocytes occurred only rarely, and infection of endothelial cells was seen primarily early after virus exposure. Only a few per cent (at most five) of the infected cells were non-neuronal and non-endothelial. These cells may be oligodendroglia or microglia. Mice infected as mid-gestation embryos with MoMuLV had infected endothelial cells, astrocytes and neurons (Table 1).

Because previous genetic studies had suggested the importance of the *env* gene in the pathogenesis of Cas-Br-E neurological disease¹⁰, tissue sections were also examined with polyclonal antibody reactive against MoMuLV or Cas-Br-E gp70. Unexpectedly, a comparison of immunocytochemical staining of adjacent spinal cord sections prepared from infected Cas-Br-E mice with the anti-Env and anti-total virus sera revealed that the two antisera gave dramatically different results. Neurons visualized by the anti-total virus antibody were not stained in adjacent sections reacted with anti-Env antibody (Fig. 1*e, f*), whereas sections of Cas-Br-E-infected spleen and thymus stained similarly with both antisera. Neurons, glia and endothelial cells in mice infected at mid-gestation with MoMuLV were stained with both antisera, as would be expected for a productive virus infection (Fig. 1*g, h*).

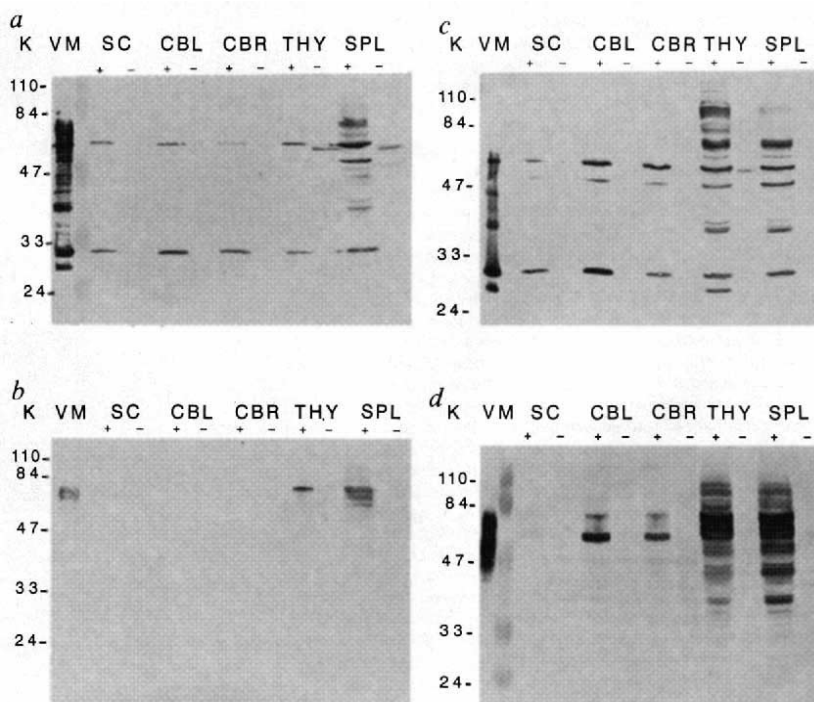
FIG. 2 Western blot analysis of tissue extracts prepared from Cas-Br-E (*a* and *b*) and MoMuLV-infected (*c* and *d*) mice and probed with anti-total virus (*a* and *c*) or anti-Env (*b* and *d*) antisera. Note that Env protein was not detected in Cas-Br-E-infected CNS but was present in other Cas-Br-E infected tissues and in all MoMuLV-infected tissues. Prominent bands with anti-total virus serum correspond to Pr65gag and p30. +, infected tissue; –, uninfected control tissue; sc, spinal cord; cbl, cerebellum; cbr, cerebrum; thy, thymus; spl, spleen; v, purified virus; m, protein relative molecular mass marker.

METHODS. Tissues were teased apart in 4 °C PBS. Cytoplasmic extracts were prepared by resuspending tissue in lysis buffer (1:5, vol cells:vol buffer; 300 mM sucrose, 1 mM EDTA, 1 mM EGTA, 20 mM Tris buffer, pH 8.0, 20 mM HEPES buffer, pH 7.3, 0.4% Nonidet-P40, 50 mM NaCl, 2 mM PMSF, 1 unit ml⁻¹ aprotinin, 5 µg ml⁻¹ chymostatin, 5 µg ml⁻¹ pepstatin and 5 µg ml⁻¹ leupeptin) and centrifuging at 400*g* for 5 min. Supernatants were diluted 1:1 in 2× Laemmli sample buffer and frozen at –70 °C. Aliquots (100 µl) of each extract were run on 12.5% polyacrylamide gels and transferred to nitrocellulose¹⁹. Nitrocellulose membranes were blocked with 5% Carnation milk, probed with goat anti-total virus (NIH81S107) or goat anti-Env (W. Schafer antibody or NIH78S225) antisera diluted in PBS containing 0.3 M NaCl and 0.1% Triton X-100 (Buffer A) for 4 h, washed in Buffer A, then incubated with rabbit anti-goat AP conjugate (Sigma, 1:2,000) for 30 min. Filters were washed with buffer A and developed

To investigate Env protein synthesis in the CNS, western blot analysis was carried out using tissue extracts prepared from mice infected with Cas-Br-E or MoMuLV and control, uninfected SWR mice. Confirming the results in Fig. 1, no Env protein was detected in the CNS of mice infected with Cas-Br-E, in contrast to mice infected with MoMuLV (Fig. 2). Viral proteins other than Env were present in tissue extracts of Cas-Br-E-infected cerebrum, cerebellum, medulla and spinal cord. The spleen and thymus of Cas-Br-E-infected mice contained all the viral proteins including Env. As expected, all viral proteins (including Env) were observed in tissues prepared from mice infected with MoMuLV at mid-gestation and probed with the same antiserum (Fig. 2). An independently prepared polyclonal antiserum to Env protein, reactive against both Cas-Br-E and MoMuLV gp70, gave identical results (data not shown). If the Env protein had been present, it should have been detectable, as the levels of other viral proteins were comparable in thymus and CNS tissue extracts.

To determine whether the *env* mRNA was present in brain and spinal cord, we examined viral transcription in infected tissues. Cas-Br-E *env* mRNA was present in CNS tissues (Fig. 3) and quantitative analysis of the amount of *env* mRNA in brain and spinal cord compared with other organs revealed no major differences. Thus, a post-transcriptional step in Env synthesis is responsible for aberrant synthesis of Env protein in the CNS.

Our studies identify the neuron as a major target for Cas-Br-E in the CNS, suggesting that spongiform degeneration could be a direct result of viral gene expression in the neuron, rather than an indirect consequence of viral infection of glia or endothelial cells^{7,10}. It was unexpected that a post-mitotic cell would be the main target for Cas-Br-E and in this respect Cas-Br-E resembles the lentiviruses, which replicate in non-dividing macrophages^{13,14}. The interaction of Cas-Br-E with neurons, however, differs in important ways from its interaction with other cells. Replication of Cas-Br-E in neurons, but not in non-neuronal cells, is abortive, by contrast with replication of the non-neurotropic MoMuLV which replicates productively in all cells. Abortive infection may explain the failure in previous



in 1 mg ml⁻¹ nitroblue tetrazolium, 0.5 mg ml⁻¹ BCIP in 50 mM diethylamine, pH 9.5 containing 10 mM MgCl₂.

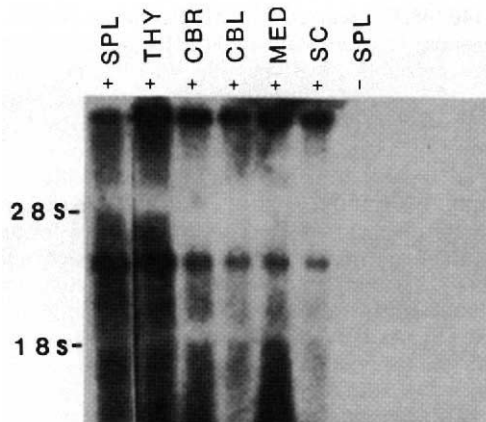


FIG. 3 Northern blot analysis of RNA from Cas-Br-E mice. +, infected tissue; -, uninfected control tissue. Abbreviations as in Fig. 2; med, medulla. The +Spl lane was exposed half as long as other lanes.

METHODS. Total RNA was isolated from tissue obtained from a 3-month-old mouse infected with Cas-Br-E neonatally by acid guanidinium-thiocyanate-phenol-chloroform extraction²⁰ and separated on 1% agarose, 2.2 M formaldehyde gels. RNA was transferred by capillary blotting onto Gene Screen Plus nylon membranes (NEN). Filters were probed with a Cas-Br-E-sequence-specific probe (*Bam*-*Taq* fragment of *env*). DNA inserts of plasmid were purified, radiolabelled with ³²P by random primer synthesis²¹ and hybridized to northern blots at a concentration of 2.5×10^6 c.p.m. ml⁻¹. Hybridizations were carried out at 47 °C in 50% formamide, 5× SSC, 2× Denhardt's and 0.01% yeast transfer RNA. After overnight hybridization, filters were washed twice at 70 °C in 2× SSC, 0.1% SDS, then washed twice in 0.2× SSC, 0.1% SDS at 70 °C, and exposed.

studies to detect mature particles in neurons of infected animals.

Although Cas-Br-E viral Gag and Pol proteins were efficiently translated from viral genomic RNA in all tissues, no Env proteins were detected in brain and spinal cord, although normal amounts of *env* mRNA were synthesized. Although these results indicate that the Cas-Br-E *env* mRNA is present, they do not rule out the possibility that a splicing error not grossly affecting mRNA size has occurred. Alternatively, neuron-specific post-translational steps in Env synthesis could be impaired, leading to aberrant Env synthesis in the CNS, resulting in accumulation of viral products detrimental to normal cell function or interfering with the processing of normal cellular glycoproteins, altering cell metabolism and/or viability.

Our results are intriguing in the light of human retroviral neurological diseases. Abortive replication of virus in neurons and/or glial cells could be involved in their pathogenesis. Similarities between human immunodeficiency virus (HIV) and human T-cell leukaemia virus (HTLV-1) neurological diseases and non-inflammatory neurodegenerative diseases of unknown aetiology (such as Alzheimer's disease and Creutzfeldt-Jacob disease) have been pointed out and a retroviral aetiology suggested^{15,16}. It may be that these diseases involve retroviruses, but because viral replication is abortive, viral infection of neurons and/or glial cells may be difficult to detect. Thus, our results give impetus to the investigation of abortive retroviral infection in the aetiology of these diseases. □

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T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis

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MULTIPLE sclerosis is thought to be an autoimmune disease of the central nervous system mediated by T cells specific for a myelin antigen^{1,2}. Myelin basic protein has been studied as a potential autoantigen in the disease because of its role as an encephalitogen in experimental autoimmune encephalomyelitis and post-viral encephalomyelitis^{3,4} and because of the presence in the blood of multiple sclerosis patients of *in vivo*-activated T cells reactive to myelin basic protein⁵. Immune involvement in multiple sclerosis has been further suggested by the association with the major histocompatibility complex class II phenotype DR2, DQw1 (refs 6-9). To define the T-cell specificity toward myelin basic protein, 15,824 short-term T-cell lines were established from multiple sclerosis subjects, subjects with other neurological diseases, and normal controls. Here we report a higher frequency of T-cell lines reactive with a DR2-associated region of myelin basic protein between residues 84-102 in patients with multiple sclerosis compared with controls. A second region, identified between residues 143-168, was recognized equally in multiple sclerosis patients and controls and was associated with the DRw11 phenotype. These DR2 and DRw11 associations were also observed among T-cell lines generated from family members of a multiple sclerosis patient. The immunodominant 84-102 peptide from myelin basic protein was both DR2- and DQw1-restricted among different T-cell lines. These results raise the possibility that this immunodominant region may be encephalitogenic in some DR2⁺ individuals.

A barrier to studying the fine specificity of reactivity to myelin basic protein (MBP) in multiple sclerosis (MS) has been the low level of reactivity in standard proliferation assays^{10,11}. To obtain the necessary number of cells for analysis, short-term T-cell lines (15,824 in total) were generated from 51 subjects by culturing peripheral blood mononuclear cells with MBP and then adding interleukins-2 and -4 (IL-2 and IL-4). IL-4 increases the growth rate of T-cell clones¹² and 2.5×10^5 - 5.0×10^5 T-line cells were generated from every well plated to perform the required analysis. On day 13, each line was tested for reactivity to MBP; reactive lines were then tested for reactivity to overlapping 20-residue oligopeptides encompassing the human MBP sequence. For MHC restriction experiments, lines reactive to

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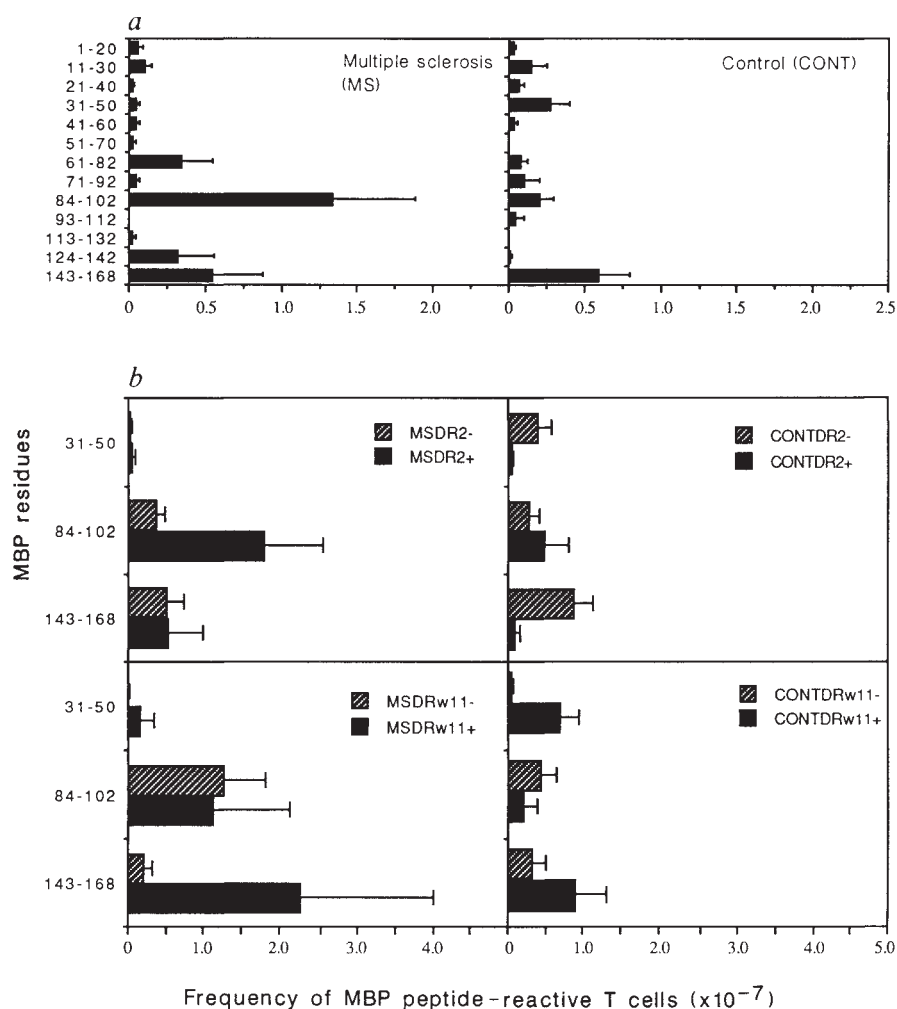
an MBP peptide were restimulated for two more cycles, first with MBP and then with the specific MBP fragment recognized by that line. In a subgroup of patients, the frequency of T cells recognizing proteolipid apoprotein (PLP), another major encephalitogenic central nervous system antigen, was investigated^{13,14}.

Frequency analysis for MBP and PLP reactivity was performed on patients with definite, early relapsing-remitting MS, subjects with other neurological diseases with central nervous system tissue damage and normal subjects, age and sex matched to the MS patients (Table 1). Although the frequency of MBP-reactive lines was slightly higher in subjects with MS compared with the other subjects, this was not statistically different. There was more reactivity to PLP in patients with MS compared with those with other neurological diseases, but this did not reach statistical significance.

Of a total of 302 lines from patients with MS that could be expanded and confirmed to react with MBP on repeated

analysis, 140 (46.4%) reacted with MBP residues 84–102. In the control groups, 11 of a total of 100 MBP-reactive T-cell lines (11.0%) recognized this MBP peptide. We calculated the actual frequency of T cells derived from the peripheral blood that reacted with each MBP peptide for each individual subject. The mean values of patients with MS and the control subjects are shown in Fig. 1a. The frequencies of peptide-specific lines from normal subjects and from other neurological disease controls were virtually identical and thus were combined for analysis. The mean frequency of T-cell lines selectively reactive to MBP residues 84–102 was higher in subjects with MS compared with controls, although there were no differences among groups in the relative stimulation indices of T-cell lines reacting to MBP peptides. Significant but less striking increases in the frequency of reactivity to MBP residues 61–82 and 124–142 were also observed in MS patients, whereas both MS and control subjects showed high frequencies of T-cell lines reactive with MBP residues 143–168.

FIG. 1 a, Mean frequency of peripheral blood T cells recognizing overlapping synthetic peptides of human MBP in 23 MS patients and 16 randomly selected controls (10 subjects with other neurological diseases of the central nervous system and 6 normal controls). For each patient, 288 wells were plated with 2×10^5 blood mononuclear cells (MNC), separated by Ficoll density gradient sedimentation, in 96-well round-bottom plates. MNC were cultured with MBP ($10 \mu\text{g ml}^{-1}$) in media consisting of RPMI 1640, 10% pooled human serum, 1% HEPES buffer, 1% penicillin/streptomycin and 2% glutamine for 13 days. On day 3 and then every 3–4 days, 5% IL-2 (ABI, Columbia, MD) and 2 U ml^{-1} of rIL-4 (Genezyme, Boston) were added to the culture. On day 13, an aliquot of each T-cell line was analysed for reactivity to MBP by placing 10,000 cells from each T-cell line with 10,000 irradiated autologous MNC as antigen-presenting cells (APC) in duplicate for 72 h in a round-bottom 96-well microtitre plate and pulsing with [^3H]-thymidine during the last 18 h of culture. APC were prepared by pulsing 10^7 APC in 1.0 ml media with or without $100 \mu\text{g MBP}$ for 1 h at 37°C . Lines positive for proliferation to MBP (defined as showing both a stimulation index of >3 and a $\Delta\text{c.p.m.}$ of >500) were re-tested 72 h later for reactivity to 13 different peptides (concentration of antigen $100 \mu\text{g peptide ml}^{-1}$) encompassing the human MBP sequence. MBP was extracted from human brain tissue, purified on a CM-52 column, and the highest relative molecular mass (M_r , 18,500) was used²⁴. MBP peptides were synthesized by Biosearch Lab Inc., (San Rafael, California) using the solid phase method and were purified by HPLC. MBP peptide sequences are as follows (single-letter amino-acid code): 1–20, ASQKRPSQRHGSKYLATAST; 11–30, GSKYLATASTMDHARHGFLP; 21–40, MDHARHGFLPRHRDTGILDS; 31–50, RHRDTGILDSIGRFFGGDRG; 41–60, IGRFFGGDRGAPKRGSGKDS; 51–70, APKRGSGKDSHHPARTAHYH; 61–82, HHPARTAHYHGSPLQKSHGRT; 71–92, SLPQKSHGRTQDENPVVHFF; 84–102, DENPVVHFFKNIVTPRTTP; 93–112, KNIVTPRTTPPSQGGKGRGLS; 113–132, LSRFSWGAEGQRPGFGYGGGR; 124–142, RPFYGGGRASDYKSAHKG; 143–168, FKGVDAGQTLKIFKLGGRD. In pilot experiments, plating 2×10^5 mononuclear cells per well gave frequencies of between 1 and 20% MBP-reactive lines. Thus according to the Poisson probability distribution, most MBP-reactive T-cell lines represent a single MBP-reactive T cell. As 2×10^5 MNC were plated for each of 288 wells per subject, the actual frequency of MBP-reactive T cells can be calculated by dividing the frequency of MBP reactive lines (Table 1) by 2×10^5 . The frequency of T cells reactive with specific MBP synthetic peptides was calculated by multiplying the frequency of MBP-reactive T cells for each subject by the proportion of lines reactive with each peptide on the second analysis for that same subject. Specifically, the frequency of circulating T cells reactive with synthetic peptide is equal



to the frequency of MBP-reactive T cells $\times$ (no. T-cell lines reactive with synthetic peptide)/(Total no. MBP reactive lines on 2nd analysis). The mean frequency $\pm$ s.e.m. for each peptide for patients with MS, normal controls and subjects with other neurological diseases were calculated. Values for both control groups were combined. b, Frequency of MBP peptide-reactive lines in relation to class II MHC expression in patients with MS and in controls. An additional 6 DR2⁺ normal controls were studied in addition to the original 16 control subjects and 23 patients with MS. Subjects with the DR2 phenotype had a higher proportion ($P=0.003$) and frequency ($P=0.045$) of T cells recognizing MBP residues 84–102. Subjects with the DRw11 phenotype had a higher frequency ($P=0.006$) of T cells recognizing MBP residues 143–168 (unpaired *t*-test).

TABLE 1 Subject characteristics and frequency analysis of MBP- and PLP-reactive lines

	Age	Sex (%) (m/f)	MHC (%)				No. antigen reactive lines/ total no. lines		Mean frequency of antigen-reactive lines (%)	
			DR2	DR4	DRw11	DQw1	MBP	PLP	MBP	PLP
Multiple Sclerosis (<i>n</i> = 23)	34.2 ± 1.4	35/65	60.9	26.1	13.0	78.2	554/7746	20/432	7.18 ± 2.38	3.34 ± 1.56
Other Neurological disease (<i>n</i> = 10)	38.7 ± 3.2	43/57	14.3	0.0	42.9	85.7	118/2880	3/384	4.10 ± 1.04	0.90 ± 0.62
Normal (<i>n</i> = 6)	30.3 ± 1.5	50/50	16.7	0.0	50.0	66.6	73/1742	ND	4.70 ± 1.58	ND
DR2 ⁺ Controls (<i>n</i> = 6)	32.0 ± 2.9	50/50	100	16.7	0.0	100	53/1728	ND	3.08 ± 2.06	ND

Patients with MS were Caucasian and had well-characterized early relapsing remitting disease with minimal disability with at least two exacerbations within the previous 24 months and positive lesions on MRI at the time of blood drawing. Subjects with other central nervous system diseases had the following diagnoses: 1–3 weeks after either cerebrovascular accident (4); brain trauma with central nervous system haemorrhage (4); metastatic brain tumour (2). The total number of lines reactive with either MBP or PLP and the total number of T-cell lines generated are shown. The frequencies of MBP- and PLP-reactive lines were calculated separately for each subject by dividing the number of MBP-reactive lines by the total number of lines generated and the mean value ± s.e.m. are given. See Fig. 1 legend for methods for T-cell cloning. ND, not determined.

TABLE 2 MHC restriction of T-cell lines reactive to MBP 84–102

[³ H]thymidine incorporation (Δ c.p.m.) of T-cell lines																	
(a) MHC phenotype of APC		Hy. 1A8			Hy. 2C9			Hy. 2E11			Hy. 2H9			Hy. 3A10			
		DR	DQw	APC	MBP	Peptide 84–102	APC	MBP	Peptide 84–102	APC	MBP	Peptide 84–102	APC	MBP	Peptide 84–102	APC	MBP
	2,7	1,3	32	21,192	10,747	83	3,263	14,991	148	18,593	30,368	169	2,797	10,444	139	6,887	24,411
	2	1	83	56	32	82	78	112	217	52,939	49,399	636	327	658	23	28	26
	4,7	2,3	32	26	53	45	55	142	37	167	81	226	258	263	306	719	915
	3	2	46	32	52	43	44	110	101	98	349	769	402	1,973	23	31	100
	3,10	1,2	35	30,737	49,144	158	25	80	36	58	42	49	54	46	42	22,823	31,121
	2,7	1,2	38	40	47	43	39	43	78	53,441	32,357	261	190	289	33	19	36
	7.w11	2,7	44	39	54	57	124	259	34	25	33	51	58	97	967	1,214	2,744
(b) Monoclonal antibodies																	
Specificity	APC alone		32			83			39			50			139		
	None		10,747			14,991			3,325			8,659			24,411		
	Control		11,375			15,322			4,131			8,156			27,363		
Anti-DR	PL8		11,051			41			31			142			25,016		
	L243		16,792			586			22			36			21,148		
	6SP4.1		19,119			405			46			92			26,412		
Anti-DQ	1A3		4,851			11,444			2,102			5,446			15,714		
	Tu22		1,189			13,442			1,073			7,661			13,488		
	Leu10		1,128			14,924			2,255			7,678			13,090		
Anti-DP	B7/21		7,917			15,922			2,337			6,689			23,452		
Anti-DR + DP	Tu35		13,606			75			21			42			27,104		

(a) Proliferation of T-cell lines using a panel of different MNC as APC are shown. Five T-cell lines reactive to MBP residues 84–102 from subject Hy were expanded by repeated cycles of stimulation with autologous irradiated MNC pulsed with synthetic MBP peptide 84–102 and examined for recognition of this region of MBP and human MBP. Briefly, 50,000 T-line cells were plated in triplicate with 50,000 irradiated APC MNC for 72 h in round-bottom 96-well microtitre plates and wells were pulsed with [³H]thymidine for the last 18 h culture. APC MNC were either cultured alone, pulsed with 100 μg ml⁻¹ synthetic peptide 84–102 (the optimal concentration of peptide to induce proliferation), or pulsed with 100 μg ml⁻¹ MBP. The average c.p.m. values for triplicate wells are shown. DR and DQw haplotypes are given and haplotypes common with the patient (top line), who was DR2, DR7, DQw1, DQw3, are in bold. (b) Inhibition of proliferation by class-specific anti-MHC monoclonal antibodies. The panel of five T-cell lines reactive with MBP residues 84–102 were plated with autologous APC MNC either alone or in the presence of APC pulsed with synthetic peptide 84–102, as above, in the presence of monoclonal antibodies (final concentration 1:100) recognizing different MHC class II gene products. Control was an irrelevant monoclonal antibody. Antibody nomenclature according to the 10th International Histocompatibility Workshop²⁵.

As previously reported^{6–9}, the DR2, DQw1 phenotype was infrequent in the controls and more common in MS patients (Table 1). An association was observed between the DR2 phenotype and both the proportion and the frequency of T-cell lines reactive to MBP residues 84–102 (Fig. 1b). To determine whether there was also this association in non-MS subjects, an additional six normal subjects with the DR2, DQw1 phenotype were investigated. An association with DR2 was also found among controls in terms of the proportion of T-cell lines reactive with MBP residues 84–102 (DR2⁺ controls, 31.0 ± 10.8%; DR2⁻ controls, 10.1 ± 0.4%). The total frequency of lines reactive with this region of MBP was, however, less than that in patients with MS (Fig. 1b). Although DQw1 is in linkage disequilibrium with

DR2, independent analysis of peptide reactivity revealed no association with DQw1 phenotype. The lack of a DQw1 association in subjects with MBP peptide 84–102 reactivity may, however, also relate to differences in DQw1 polymorphisms that were not examined in the present study.

The DRw11 phenotype was more common in controls than in subjects with MS (Table 1). Its expression was associated with a higher frequency of lines reactive to MBP residues 143–168 in both MS patients and controls (Fig. 1b). Reactivity to MBP residues 31–50, which was predominantly observed in controls, was also associated with DRw11. Other MHC associations were not found.

It was important to determine the MHC restriction of the

TABLE 3 Relationship between MHC expression and frequency of T cells reactive to immunodominant MBP epitopes in family with DR2 and/or DRw11 phenotypes

Patient	P1	P2	S1	S2	S3
DR2	DR4		DR4	DR4	
DQw1	DRw53	DR2	DRw53	DRw53	DR2
	DQw3	DQw1	DQw3	DQw3	DQw1
DRw11	DRw11	DRw6	DRw6	DRw6	DR4
DRw52	DRw52	DRw52	DRw52	DRw52	DRw53
DQw1	DQw1	DQw1	DQw1	DQw1	DQw3
MBP peptide					
84-102	49	4	6	1	7
143-168	41	0	3	2	2

The family members of an MS patient expressing the DR2, DQw1; DRw11, DRw52, DQw1 class II MHC haplotypes were examined for the frequency of T-cell lines reactive with MBP residues 84-102 and 143-168. MNC (2×10^5) in each of 288 wells (three 96-well round-bottom plates) were cultured with MBP ($10 \mu\text{g ml}^{-1}$) as outlined above for each subject. On day 16, each T-cell line was analysed for reactivity to synthetic peptides corresponding to MBP residues 84-102 and 143-168. The number of lines reactive with each peptide (SI > 3, $\Delta\text{c.p.m.} > 500$) generated per subject are shown. The actual stimulation indices were generally >20. P1 and P2, parents; S1-S3, siblings.

T-cell lines reactive with the immunodominant MBP epitope. Monoclonal antibody blocking studies of five T-cell lines reactive with MBP residues 84-102 suggested that both DR and DQ molecules could function as restricting elements. Among clones blocked by anti-DR monoclonal antibody, clone 2E11 proliferated in response to MBP residues 84-102 with the panel of DR2⁺ antigen-presenting cells (APC) whereas 2C9 and 2H9 proliferated only with autologous APC (Table 2). The recognition of peptide by clones 1A8 and 3A10, which were partially blocked by anti-DQ monoclonal antibodies, was restricted to APC from the responder and one of two APC donor subjects expressing DQw1.

To investigate the relationship between MHC expression and frequency of T-cell reactivity to immunodominant MBP epitopes, a family with one afflicted sibling of both DR2 and DRw11 phenotypes was studied (Table 3). A total of 1,728 individual T-cell lines were generated from both parents and four siblings and the number of lines reactive with either MBP peptide 84-102 or 143-168 was determined. The DR2⁺, DRw11⁺ patient had a high frequency of T-cell lines reactive to both MBP residues 84-102 and 143-168. The DRw11⁺ parent preferentially recognized MBP residues 143-168, whereas the DR2⁺ parent preferentially recognized MBP residues 84-102. The frequency of MBP peptide reactive lines, however, was lower than that of the patient. One sibling was DR2⁺ and preferentially recognized MBP residues 84-102. Of two HLA-identical siblings (DR4, DQw3/DRw6, DQw1), one preferentially recognized MBP peptide 84-102. Although DR4 or DQw1 may also restrict recognition of MBP residues 84-102, other factors such as inherited T-cell receptor polymorphisms may have influenced T-cell reactivity to the MBP autoantigen in one of the DR4, DQw3/DRw6, DQw1 siblings. In this regard, DR4 was also more frequent in our series of MS patients compared with control subjects. This family linkage analysis suggests that optimum recognition of immunodominant MBP epitopes requires specific class II MHC alleles both in patients with MS and controls. In total, these studies indicate that although control subjects expressing DR2 seem preferentially to recognize the same MBP determinant as do DR2⁺ MS patients, their clonal frequency in the blood is less than that of patients with MS.

Previous studies using peripheral blood have suggested there is a response to a number of MBP regions, in particular 83-96, which contains an epitope that overlaps with the immunodominant 84-102 MBP region identified here¹⁵. In those studies, however, the standard 7-day-proliferation assay did not detect peptide reactivity in controls. The presence of T cells reactive with MBP residues 84-102 in DR2⁺ normal subjects suggests that other immune mechanisms exist to prevent the activation of circulating autoreactive T cells. Although in the present study MBP-reactive T cells could be generated by culture *in vitro* from controls to the same degree as patients with MS, recent work indicates that MBP-reactive T cells are activated only in patients with MS and not in controls⁵. Other factors

involved in the development of autoimmune diseases may relate to the altered systemic immunoregulation seen in MS as evidenced by decreased levels of antigen-nonspecific suppression^{16,17}.

In experimental autoimmune encephalomyelitis (EAE), a single immunodominant region of MBP influenced by class II MHC has been observed among different rat and mouse strains¹⁸⁻²¹ and these peptides are the major encephalitogen if the animal strain is susceptible to EAE²². In determining the relationship between MHC and encephalitogenic immunodominant regions of MBP, the animal need not have had EAE. Unprimed Lewis rat lymph-node cells selectively react with immunodominant MBP residues 48-88 and these T-cell clones are encephalitogenic²³. The present experiments in humans define immunodominant regions of human MBP that are linked to a particular MHC. As the DR2, DQw1 phenotype is most common in patients with MS⁶⁻⁹, the T-cell reactivity to MBP residues 82-102 in association with this phenotype may be analogous to the encephalitogenic T cells defined in EAE.

To show that MS is a cell-mediated autoimmune disease analogous to EAE, certain criteria can be proposed. First an association should exist between an immunodominant region of the presumed autoantigen and disease-associated class II MHC haplotypes. Second, there should be an increase in frequency of T cells that react with this immunodominant epitope. Finally, the course of the disease must be altered by elimination of autoreactive T cells or by inducing immune tolerance to the autoantigen identified in the first two criteria. This final condition implies that *in vitro* experiments on their own cannot prove the association of an autoantigen with a disease and instead clinical trials are necessary.

Our results have begun to address the first two criteria, showing an association between immunodominant regions of human MBP with the DR2 class II MHC phenotype and an increased frequency of T cells recognizing the MHC-linked immunodominant epitope of the MBP autoantigen in MS. In addition, PLP might also serve as an autoantigen. If MBP is a target autoantigen in MS, our results imply that an immunodominant region of MBP, between residues 84-102, is an encephalitogenic region in some DR2⁺ individuals. In non-DR2 individuals other immunodominant MBP epitopes or other antigens may be associated with the disease.

Note added in proof: Since submitting our article, two works have come to our attention demonstrating similar immunodominant regions of human MBP: Martin, R. *et al.*, *J. Immunol* (in the press); Pette, M., *Proc. natn. Acad. Sci. U.S.A.* (in the press). □

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Activated CD8 binding to class I protein mediated by the T-cell receptor results in signalling

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THE CD8 glycoprotein of T cells binds nonpolymorphic regions of class I major histocompatibility complex proteins on target cells^{1,2} and these interactions promote antigen recognition and signalling by the T-cell receptor³⁻¹¹. Studies using artificial membranes indicated that effective CD8/class I interaction is critical for response by alloantigen-specific cytotoxic T lymphocytes when class I protein is the only ligand on the antigen-bearing surface^{10,11}. But significant CD8-mediated binding of cytotoxic T lymphocytes to non-antigenic class I protein could not be detected in the absence of the alloantigen¹². These apparently contradictory findings indicate that CD8 binding to class I protein might be activated through the T-cell receptor and the results reported here demonstrate that this is the case. Treatment of cytotoxic T lymphocytes with soluble anti-T-cell receptor antibody activates adhesion of the cytotoxic T lymphocytes to class I, but not class II proteins. The specificity of this binding implies that it is mediated by CD8 and blocking by anti-CD8 antibodies confirmed this. Furthermore, binding of CD8 to class I protein resulted in generation of an additional signal(s) necessary to initiate response at low T-cell receptor occupancy levels.

Purified class I alloantigen can be immobilized on plastic and stimulates T-cell receptor (TCR) triggered degranulation by cloned cytotoxic T lymphocytes (CTL)¹¹. Specific binding of ⁵¹Cr-labelled CTL to the immobilized alloantigen can be measured (Fig. 1). Roughly 15-20% of C11 CTL, specific for H-2K^b, were bound following a 2-h incubation at 37 °C with immobilized K^b antigen. Nonspecific binding in wells blocked with fetal calf serum (FCS) or having immobilized class II (I-A^k) protein was only ~2%. No binding above this background level was found when non-antigenic class I proteins (H-2D^b or D^d) were immobilized. In numerous experiments, specific binding of 15-30% of the CTL to alloantigen has been observed, consistent with studies examining target-cell binding by cloned CTL (refs 13, 14).

To determine if CTL binding to irrelevant class I protein could be activated by the TCR, binding was measured after

treating CTL with anti-TCR monoclonal antibody F23.1 in solution. Soluble F23.1 stimulated CTL binding to immobilized class I, but not to class II major histocompatibility complex (MHC) proteins (Fig. 1). Binding occurred equally well to the class I protein borne by the CTL (D^d) or to third-party class I protein (D^b) and was comparable to that obtained to alloantigen (K^b) in the absence of soluble anti-TCR monoclonal antibody. Stimulation of binding was specific for anti-TCR monoclonal antibody. Addition of irrelevant antibodies or antibodies specific for several other CTL surface proteins including H-2K^k, CD8 or Thy-1 did not trigger binding to class I proteins (data not shown). Binding to irrelevant class I proteins was detectable within minutes of adding anti-TCR antibody and centrifuging the cells into the wells, increased to 15-30% over the next hour, and then remained stable. Similar kinetics were observed for binding to alloantigen in the absence of soluble anti-TCR antibody (data not shown).

Neither soluble anti-TCR monoclonal antibody nor immobilized class I protein alone (other than the alloantigen) trigger CTL degranulation. Degranulation is triggered, however, by the combination of soluble anti-TCR antibody and immobilized class I proteins. As for binding, triggering of degranulation is

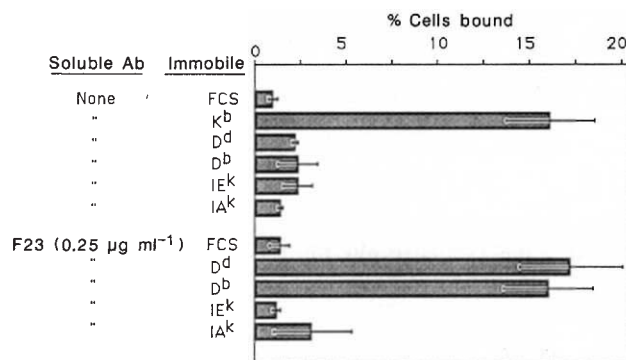
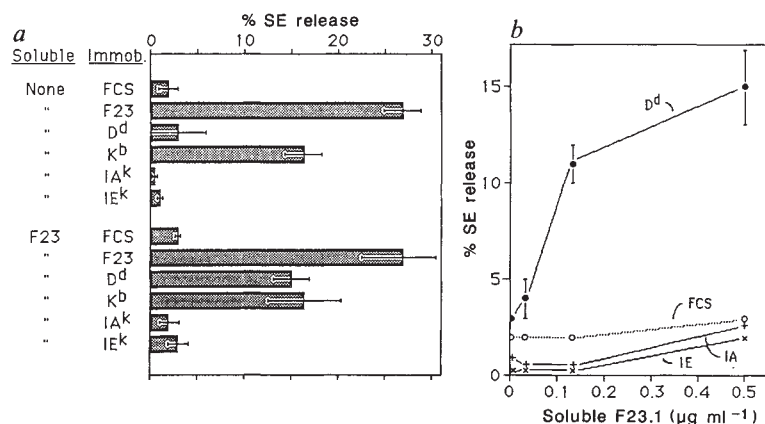


FIG. 1 Soluble anti-TCR monoclonal antibody triggers CTL binding to irrelevant class I protein. The indicated MHC proteins were immobilized (0.25 µg per well) and the wells blocked using 2% FCS. Binding by C11 was determined as described below using a 2.5-h incubation at 37 °C. Where used, soluble anti-TCR monoclonal antibody (F23.1) was added to the CTL suspension (final concentration 0.25 µg ml⁻¹) immediately before initiating the binding assay. For this experiment, total ⁵¹Cr incorporation was 26,428 c.p.m. per 10⁵ cells, and spontaneous release was 815 c.p.m. per 10⁵ cells.

METHODS. Cloned CTL: The cloned CTL lines used included C6, C11, and C13. Characterization and maintenance of these have been described¹¹. All were derived from B10.BR × B10.D2F1 (H-2^k × H-2^d) responder mice and were specific for the H-2K^b alloantigen. MHC protein purification and immobilization: MHC class I and II proteins were purified by monoclonal antibody affinity chromatography and immobilized on plastic as previously described^{11,31}. In brief, purified antigen in 0.5% deoxycholate was diluted in PBS, pH 7.2, to a final concentration of 1 or 2 µg ml⁻¹, added to wells of a plastic microtitre plate (Falcon 3912 Flexible Assay Plate), allowed to bind 1.5 h at 25 °C and unbound protein then removed. Then 2% FCS was added to the wells for 0.5 h to block unoccupied sites. Assay of CTL binding: CTL were labelled by incubating for 1 h at 37 °C using 100 µCi Na⁵¹Cr per 10⁷ cells. Cells were then washed and resuspended in RPMI medium containing 2.5% newborn calf serum (10⁶ per ml). Binding was initiated by adding 10⁵ cells per well in 0.1 ml and centrifuging at 1,200 r.p.m. for 1 min. Plates were then incubated for 1-2.5 h at 37 °C in 5% CO₂. After this, 0.1 ml PBS per well was added, plates placed on an ice water bath for 10 min and unbound cells removed by pipetting 10 times with a Pipetman (Gilson, Inc.). Well bottoms were then cut off and radioactivity levels determined. Spontaneous release of ⁵¹Cr was determined for cells incubated under identical conditions. Binding was calculated as percentage cells bound = 100 × (c.p.m. bound)/(total c.p.m. - spontaneous c.p.m.). Visual counting confirmed that this procedure provided an accurate measurement of bound cells. Antibodies used: the monoclonal antibodies used in this study were F23.1 anti-TCR, 145-2C11 anti-T3, 53-6.7 anti-Lyt-2 (CD8), YTS 169.4 anti-Lyt-2, 31-68.8 anti-Lyt-2, HO-13-4 anti-Thy-1.2, 11-4.1 anti-H-2K^k and anti-SASD, a murine IgG specific for a chemical hapten. The sources and purification of these antibodies have been described previously¹¹.

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FIG. 2 CD8 binding to class I protein provides signalling necessary for the degranulation response. *a*, C11 CTL (10^5 per well) were added to wells containing the indicated immobilized protein (Immobil.). When used, F23.1 anti-TCR monoclonal antibody was added to the CTL suspension at a final concentration of $0.5 \mu\text{g ml}^{-1}$ immediately before CTL were added to the wells. Cells were centrifuged and plates incubated for 2.5 h at 37°C . After this, aliquots of supernatant were removed and assayed for serine esterase (SE) activity as previously described¹¹. *b*, Triggering of serine esterase release was determined as described above, except the concentration of soluble F23.1 monoclonal antibody was varied as indicated. Wells contained immobilized H-2D^d (●), I-A^k (+), I-E^k (×), or FCS (○).



specific for class I (H-2D^d) and not class II (I-A^k) proteins (Fig. 2) and is dependent on the level of anti-TCR monoclonal antibody present (Fig. 2b). Additional experiments have shown that D^d, D^b and K^k class I proteins are all effective and that response occurs only when the soluble antibody is directed against the TCR. Thus, binding of CTL to class I protein, triggered by the TCR, results in generation of an additional signal(s) for the response.

The binding and class I-dependent response detected in these experiments is probably mediated by CD8; the alternative is that CTL have an additional, unidentified receptor that specifically binds class I conserved determinants. Although TCR may have a low affinity for self-MHC, there is no apparent contribution from this, as class I proteins are bound and stimulate equally well whether or not they are the same as those present on the responder cells. Further evidence that CD8 mediated the binding was that soluble anti-CD8 antibody blocked binding to class I protein triggered by soluble anti-TCR antibody (Fig. 3), although control antibodies including anti-Thy-1 did not (data not shown). Anti-CD8 antibody also effectively blocked degranulation in response to the combination of soluble anti-TCR antibody and class I protein. By contrast, immobilized anti-TCR antibody stimulated a comparable level of degranulation but the response was only marginally affected by anti-CD8 antibody (Fig. 3), indicating that little or no anti-CD8-induced negative signalling was occurring under the conditions used.

These results indicate that perturbation of the TCR generates a signal to activate CD8 binding to class I protein. Several phosphorylation events occur on T-cell triggering, including phosphorylation of the CD8 protein¹⁵. Protein kinase inhibitors K252a and staurosporine inhibited anti-TCR-triggered binding to class I protein (Fig. 4) with 50% inhibitory concentrations (IC_{50} s) similar to those found in studies directly examining inhibition of kinase activities. In addition to inhibition of binding, degranulation was inhibited (data not shown). Thus a

kinase, possibly protein kinase C, is likely to be involved in TCR-dependent activation of CD8 binding. A kinase may also be involved in the signalling resulting from CD8/class I binding. CD8 is associated with $p56^{\text{lck}}$ protein-tyrosine kinase¹⁶, which can phosphorylate the ζ -subunit of the TCR complex. If CD8 binding to class I protein activates $p56^{\text{lck}}$ to phosphorylate ζ -chain, as seems to occur on CD4 cross linking¹⁷, this might modify signalling occurring through the occupied TCRs and result in the initiation of response. A mechanism involving CD8 feedback to the TCR complex is compatible with the fact that a response can be triggered through the TCR without a CD8 ligand, as occurs with immobilized anti-TCR monoclonal antibody (Figs 2 and 3; refs 11, 18) or in CTL lacking CD8 (ref. 19).

Synergistic signalling through anti-TCR and anti-CD8 or anti-CD4 antibodies has been used to suggest that the most effective signalling involves a trimolecular complex, with TCR and CD8 (or CD4) interacting with the same MHC protein²⁰⁻²⁴. This mechanism could account for the fact that, in general, class I-restricted cells carry the CD8 antigen and class II-restricted cells carry the CD4 antigen. Formation of trimolecular complexes is unlikely in the experiments described here, as the TCR and CD8 do not use the same ligand. However, this does not eliminate the possibility that a physical association between TCR and CD8 is critical, and that this occurs even when the two receptors interact with different ligands.

Although CD8/class I binding by CTL was not observed in the absence of a TCR stimulus (Fig. 1), chinese hamster ovary (CHO) cells transfected with the CD8 α -chain gene bind to class I-bearing cells¹. Binding by these TCR-negative cells was observed in the absence of any stimulus. But binding was observed only when very high surface levels of CD8 were expressed in these transfectants. Thus, it seems likely that CD8 has some affinity for class I protein in the unactivated state, but that this is detectable only at high surface densities of CD8. By contrast, with the CD8 surface density normally present on T

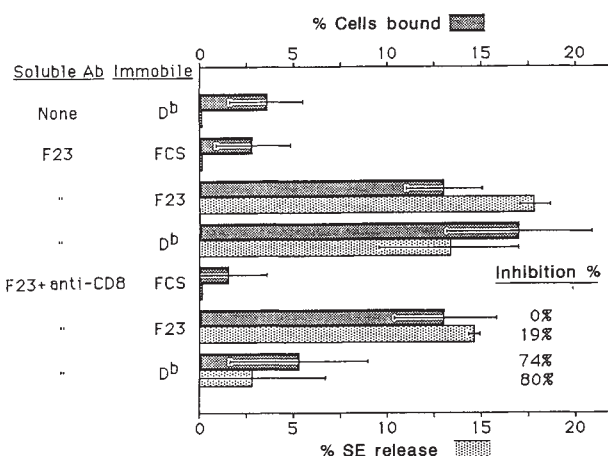


FIG. 3 Anti-CD8 antibody blocks TCR-activated binding and response to non-antigenic class I protein. The ^{51}Cr -labelled clone 11 CTL (10^5 per well) were incubated for 20 min at room temperature alone or with soluble F23.1 anti-TCR monoclonal antibody ($0.25 \mu\text{g ml}^{-1}$). Where used, YTS 169.4 monoclonal antibody (as ascites) was then added at a 1:900 dilution. YTS 169.4 is a rat monoclonal antibody that recognizes a monomorphic murine Lyt-2 determinant³². Cells were then added to wells bearing the indicated immobilized protein, centrifuged and the plates incubated for 3 h at 37°C . After this, aliquots of supernatant were removed and assayed for serine esterase (SE) activity¹¹. Binding was then determined in the same wells as described (Fig. 1). Parallel comparisons of unlabelled and ^{51}Cr -labelled CTL have shown that ^{51}Cr -labelling has no effect on the degranulation response (data not shown). H-2D^b antigen used at $0.2 \mu\text{g}$ per well.

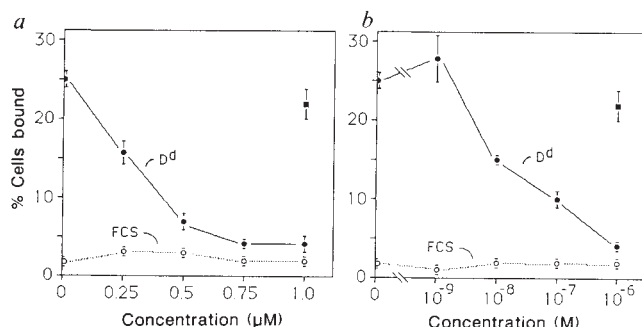


FIG. 4 Inhibition of TCR-activated CD8/class I binding by kinase inhibitors. Soluble F23.1 anti-TCR monoclonal antibody ($0.25 \mu\text{g ml}^{-1}$) was added to ^{51}Cr -labelled C11 CTL immediately before initiating binding to wells containing H-2D^d ($0.1 \mu\text{g}$ per well; ●) or FCS (○). K252a (a) or staurosporine, (b) (Calbiochem) were added in dimethylsulphoxide (DMSO) ($\leq 1\%$ final concentration) to CTL 15 min before initiating binding. DMSO was added to solvent controls (■; 1% final concentration). Binding was for 2.5 h at 37°C . Total ^{51}Cr incorporation was 40,298 c.p.m. Spontaneous release was determined at all drug concentrations and did not vary significantly. The average spontaneous release for all samples was 1,308 c.p.m.

cells, binding is only detected on activation through the TCR (Fig. 1).

Possible mechanisms by which TCR signalling might activate CTL binding mediated by CD8 include: rapid translocation of an internal pool of CD8 to the cell surface (preliminary results indicate that this does not occur on addition to CTL of soluble anti-TCR monoclonal antibody); induced microclustering of CD8, a mechanism implicated for PMA-activation of binding by C3bi receptors²⁵; or conversion of CD8 from a low-affinity to a high-affinity state on TCR signalling. An example of the latter is provided by several hormone and growth-factor receptors whose affinity for ligand is modulated by phosphorylation²⁶. These mechanisms are not mutually exclusive and more than one could contribute to activation of CD8 binding.

Results reported here demonstrate that CD8/class I interactions represent a TCR-activated adhesion-signalling system. Another adhesion system important in T-cell responses, that of LFA-1 and its I-CAM 1 and 2 ligands^{27,28}, is activated by crosslinking the TCR^{29,30}. Whether the binding of LFA-1 to I-CAM mediates signalling events that contribute to TCR-dependent responses is not known. Adhesion-strengthening mechanisms triggered by TCR recognition of antigen, but distinct from TCR-antigen bonds, have been proposed, and Martz²⁸ has discussed in detail how such mechanisms can account for the properties of CTL/target cell interactions. It now seems that both LFA-1/I-CAM and CD8/class I represent such mechanisms, with at least the CD8/class I interaction also resulting in generation of a signal(s) for response. □

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Small G proteins are expressed ubiquitously in lymphoid cells and do not correspond to Bcl-2

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THE *bcl-2* gene is consistently associated with t(14; 18) chromosomal translocations^{1–6} observed in a large fraction of human B-cell lymphomas^{7–8}. The t(14; 18) translocation results in deregulated expression of the *bcl-2* gene⁵ and synthesis of inappropriately high levels of the Bcl-2 protein^{9,10}. Gene transfer studies suggest a role for Bcl-2 in cell survival^{11,12}, growth enhancement^{13,14} and oncogenic transformation^{11,15}. To test the suggestion that GTP-binding by Bcl-2 may mediate its biological effects¹⁶ we characterized the GTP-binding proteins in lymphoid cells expressing Bcl-2. Expression of several small GTP-binding proteins was found to be ubiquitous and did not vary with levels of Bcl-2. By using immunological, electrophoretic and cell-fractionation techniques, we separated Bcl-2 from G proteins of small relative molecular mass (M_r) and showed that it is incapable of binding GTP. Our results show that small M_r G proteins are widely expressed in lymphoid cells and that Bcl-2 is not a novel member of this GTP-binding protein family.

The prevalence of small M_r G proteins in cells of the B-cell lymphoid lineage was investigated using a GTP-binding assay^{17,18} that identifies the small M_r GTP-binding proteins but not the large G proteins with an $\alpha\beta\gamma$ subunit structure¹⁹. The cell lines examined corresponded to different stages of B-cell maturation and included pre-B cells (leukaemia line 697), mature B cells (lymphoma lines SU-DHL-4, DUL-5 and FL18) and plasma cells (murine plasmacytoma line J558L). In all lines, four small M_r GTP-binding proteins similar to those observed in NIH 3T3 cells were detected (Fig. 1). A fifth GTP-binding protein of M_r 21,000 (21K), recognized by the Ras11 monoclonal antibody²⁰, was also found in some lines.

In spite of the uniform expression of small M_r G proteins in these B-cell lymphoma cell lines, there were marked differences in Bcl-2 expression (Fig. 1), which ranged from very high levels in FL18 to undetectable levels in SU-DUL-5. There was also no obvious increase in GTP-binding activity or additional GTP-binding proteins in cell lines that overexpressed Bcl-2, or in the *bcl-2*-transfected EP5 cells compared with their non-transfected counterpart J558L (Fig. 1). These observations suggested no correlation between levels of GTP-binding and Bcl-2 expression.

The GTP-binding potential of Bcl-2 was more directly assessed after immunoprecipitation from total cellular extracts. Bcl-2 was the only protein specifically precipitated with anti-Bcl-2 antibodies as compared with pre-immune serum (Fig. 2a). The immune pellets and supernatant fractions were analysed

for the presence of Bcl-2 (by anti-Bcl-2 western blot analysis) and for GTP-binding activity (by exposing the nitrocellulose-immobilized proteins to [α - 32 P]GTP). Bcl-2 was found exclusively in the immune pellets, and had been efficiently cleared from the supernatant fractions under these conditions (Fig. 2b, lower panel). By contrast, no GTP-binding proteins were detected in the immune pellets (Fig. 2b, upper panel) as they remained completely in the supernatant fractions. These results showed that, using highly specific anti-Bcl-2 antibodies, Bcl-2 can be completely dissociated from the small M_r G proteins present in B lymphocytes, further suggesting that Bcl-2 does not bind GTP under the conditions used.

It is unlikely that the lack of observed GTP-binding by Bcl-2 immunoprecipitates (which contrasts with results reported earlier¹⁶) resulted from inactivation of the protein, as the GTP-binding activity of the small M_r G proteins remained intact following incubation with our antiserum. Furthermore, Ras immunoprecipitated from FL18 cells with the Ras11 antibody could renature to bind GTP under our experimental conditions (unpublished observations). Low levels of GTP-binding by Bcl-2 in our immunoprecipitates were ruled out by prolonged overexposure of the autoradiographs (data not shown). Finally, a recombinant Bcl-2 protein overexpressed at high levels in *Escherichia coli*¹⁰ failed to bind GTP in conditions in which the recombinant Ras protein binds²¹.

Bcl-2 has a predicted M_r of 26K but migrates in SDS-PAGE with a lower apparent M_r of ~24K (ref. 10). So it seems to co-migrate in SDS-PAGE with an ubiquitous small M_r G protein of B cells. We found electrophoretic conditions (Fig. 3a), however, in which the Bcl-2-reactive band on western blot analysis migrated in a slightly different position from this non-Bcl-2 small M_r G protein. Whereas the apparent M_r of Bcl-2 is 24K, the apparent M_r of the co-migrating GTP-binding protein was estimated to be 23.5K.

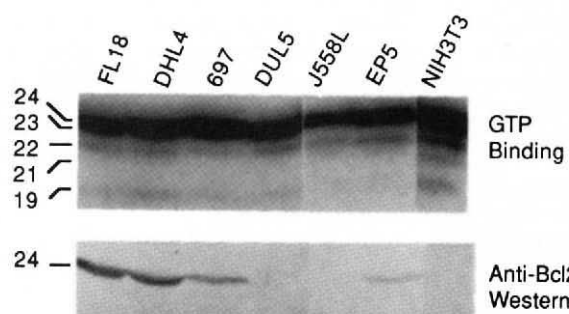


FIG. 1 Expression of Bcl-2 and low M_r GTP-binding proteins in various lymphoid and nonlymphoid cell lines. Upper panel, binding of [α - 32 P]GTP to proteins from total cellular extracts. Lower panel, western blot of total cellular proteins using anti-Bcl-2 antibodies. Cell extracts were prepared from human B-cell lymphoma cell lines FL18, DHL4 and DUL5; human pre-B leukaemia cell line 697; mouse lymphoid cell line J558L and its Bcl-2-transfected counterpart EP5; and nontransfected NIH 3T3 cells. M_r values were determined by comparison with prestained protein standards electrophoresed in the gels.

METHODS. Cells were lysed over 30 min in buffer containing 50 mM HEPES, 150 mM NaCl, 0.5% Triton X-100, and 1 mM PMSF. Cell lysates were centrifuged at 1,000 r.p.m. for 6 min at 4 °C and extracts (5×10^6 cell equivalents per lane) prepared for 11.5% SDS-PAGE by boiling 5 min in sample buffer (0.1 M Tris-HCl, pH 6.8, 1% SDS, 0.1 M DTT). Proteins were electro-transferred to nitrocellulose using transfer buffer (50 mM Tris-HCl pH 7.4, 40 mM glycine, 1 mM SDS, 20% methanol). For GTP binding the membrane was incubated overnight at 4 °C (or 30 min at 25 °C) in binding buffer (50 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 0.3% Tween-20, 0.5 mM EDTA) followed by 1-h incubation at 37 °C (or 25 °C) in binding buffer plus 0.05 mCi [α - 32 P]GTP (10 mCi ml⁻¹, Amersham 3,000 Ci mmol⁻¹). The blot was washed three times (5 min each) in binding buffer at 4 °C (or 25 °C), dried, and autoradiographed. The membrane was then subjected to western blot analysis as previously described using anti-Bcl-2 antibodies¹⁰.

In addition, cellular fractionation studies showed that the small GTP-binding proteins are distributed differently among various subcellular fractions compared with Bcl-2. The 23.5K and 23K small M_r G proteins were partly soluble and equally distributed between the cytosol and membrane fractions (Fig. 3b, upper). Bcl-2, on the other hand, was not present in the cytosol and was distributed between the nuclear and membrane fractions (Fig. 3b, lower), consistent with its properties as an insoluble membrane-bound protein predominantly localized to the rough endoplasmic reticulum and the nuclear envelope¹⁰.

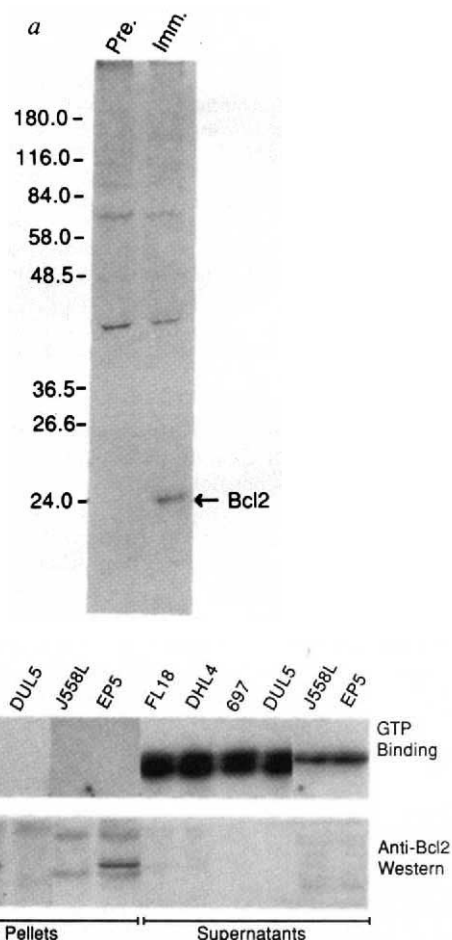


FIG. 2 Bcl-2 immunoprecipitation and GTP-binding. **a**, Immunoprecipitation of Bcl-2 from metabolically labelled FL18 cells. Pre., immunoprecipitation performed using pre-immune rabbit serum; Imm., immunoprecipitation with anti-Bcl-2 immune serum. **b**, GTP-binding (upper panel) and anti-Bcl-2 western blot analysis (lower panel) of proteins precipitated (Immune pellets) or remaining in the supernatant fraction (Supernatants). M_r standards were those described in Fig. 1.

METHODS. **a**, FL18 cells were metabolically labelled with [35 S]methionine for 6 h in methionine-free DME medium (15 mCi ml⁻¹, Amersham >1,000 Ci mmol⁻¹) and washed twice in cold PBS. Cell extracts were prepared as in Fig. 1 and immune complexes were formed overnight at 4 °C by incubating cell lysates with rabbit polyclonal serum highly specific for Bcl-2 as described elsewhere^{10,30}. Immune complexes were collected on Sepharose protein-A beads (Pharmacia) at 4 °C for 2 h. Immune pellets were washed sequentially with two SDS washes (Fig. 1 lysis buffer with 0.1% SDS and 0.1% Triton X-100), one NaCl wash (lysis buffer with 1 M NaCl and 0.1% Triton X-100), and one SDS wash, and prepared as in Fig. 1 for 10% SDS-PAGE. **b**, Immunoprecipitation and sample preparation of unlabelled cellular proteins was carried out as described above; immune pellets and corresponding supernatant fractions were electrophoresed on 11.5% SDS-PAGE gels except for J558L and EP5 which were electrophoresed on 7.5–11.5% gradient SDS-PAGE gels. Proteins were transferred to nitrocellulose filters and subjected to GTP-binding analysis and anti-Bcl-2 immunoblotting as described in Fig. 1.

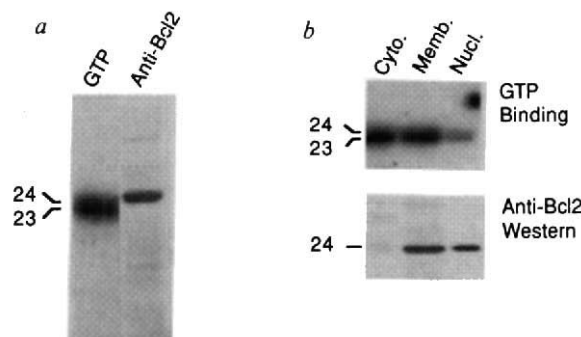


FIG. 3 Physical and biochemical fractionation of Bcl-2 and small M_r G proteins in lymphoid cells. *a*, Migration differences of Bcl-2 and small M_r GTP-binding proteins on SDS-PAGE gels. Total cellular proteins were fractionated by 11.5% SDS-PAGE and a single gel lane was subjected sequentially to GTP-binding (lane GTP) and anti-Bcl-2 western blot (anti-Bcl-2) analyses. Western filter and corresponding autoradiograph were aligned to show small but reproducible differences in migration for Bcl-2 and small M_r G proteins of 24K. *b*, Cellular fractionation demonstrating subcellular distributions of Bcl-2 and small M_r G proteins. Upper panel, GTP-binding analysis; lower panel, subsequent western blot analysis of the same filter. Cyto., soluble cytoplasmic fraction; Memb., cytoplasmic membrane fraction; Nucl., nuclear fraction. METHODS. *a*, FL18 cell extract was prepared as described (Fig. 1). *b*, Cell fractionation was performed as previously described¹⁰. Samples were run on 11.5% SDS-PAGE and subjected to GTP-binding and anti-Bcl-2 western blot analyses (Fig. 1, methods).

The above observations indicate that Bcl-2 is not one of the small M_r G proteins expressed ubiquitously in the lymphoid cell lines investigated here. The possibility that Bcl-2 binds GTP under other conditions seems unlikely as we have been unable to detect significant binding of GTP in Bcl-2 immune pellets that have not been subjected to denaturing conditions (unpublished observations). These results are consistent with the absence of significant sequence similarity of Bcl-2 with GTP-binding proteins, particularly in the highly conserved region of the 'phosphate-binding' consensus sequence (Gly-X-X-X-Gly-Lys) (ref. 22). Previous study of potential GTP binding by Bcl-2 (ref. 16) did not account for the ubiquitous small- M_r G proteins and may have been hampered by the reduced specificity of anti-peptide antibodies as reported by others²³. The small M_r G proteins that we have observed in B lymphocytes may correspond to those described previously in other haematolymphoid cells. The 23.5K and 23K proteins seem similar to those in platelets and neutrophils¹⁷⁻¹⁸, and one may correspond to the *ral* gene product²⁴. The 19K GTP-binding protein most probably is Rho (ref. 25). A 22K GTP-binding protein has also been found in platelets and probably represents the protein variously called Smg21, Krev or Rap1²⁶⁻²⁹. As none of the small M_r GTP-binding proteins in B lymphocytes corresponds to Bcl-2, the biochemical role of this proto-oncogenic protein remains to be determined. □

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The *c-ets* proto-oncogenes encode transcription factors that cooperate with c-Fos and c-Jun for transcriptional activation

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CELL transformation by oncogenes leads to changes in gene expression. A key event in this process seems to be activation of the transcription factors AP-1 and PEA 3 (refs 1-5). Their synergistic activities are required for efficient activation of transcription from different promoters by many different oncogenes, serum growth factors and the tumour promoter TPA^{4,5}. We show here that the products of the *ets-1* and *-2* proto-oncogenes, whose biological function was previously unknown⁶⁻¹¹, are transcription factors that activate transcription through the PEA 3 motif. The p68^{c-ets-1} protein specifically binds to DNA and contains a transcriptional activation domain. The *ets*-like gene family therefore seems to encode a new family of transcription factors, apparently unrelated to other transcription factors. The p68^{c-ets-1} protein cooperates with c-Fos and c-Jun (components of AP-1) for activation of transcription from the oncogene-responsive domain of the polyoma enhancer, indicating that combined activity of all three oncoproteins could be involved in the response of cells to growth stimuli.

A 14-base pair (bp) sequence from the polyoma (Py) enhancer (Fig. 1, oncogene-responsive domain) mediates transcriptional activation by serum growth factors, the tumour promoter TPA (12-*O*-tetradecanoylphorbol-13-acetate) and several oncogenes⁴. We found that expression of p68^{c-ets-1} protein in LMTK⁻ fibroblasts efficiently activated transcription through this 14-bp sequence (~20-fold; Fig. 1b, lanes 2, 3, 5, 6, 10, 12). Mutations in the PEA 3 motif of this element abolished p68^{c-ets-1} activation (four tandem copies of sequences with mutations M1, M3 or M6; Fig. 1c, lanes 2-4, 9-11). Mutations in the AP-1 motif did not hamper efficient activation (four tandem copies of sequences with mutations M7, M4 or M5; Fig. 1c, lanes 5-7, 12-14). Expression of p68^{c-ets-1} activated transcription through a PEA 3 motif multimer (~10-fold; Fig. 1b, lanes 7, 10, 9, 12), demonstrating that the PEA 3 motif is sufficient for transactivation by p68^{c-ets-1}.

To investigate whether p68^{c-ets-1} binds to the PEA 3 motif, p68^{c-ets-1} was synthesized in rabbit reticulocyte lysates and used

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for gel-retardation experiments with a PEA 3 oligonucleotide probe. A retarded complex that was absent from control extracts was observed (Fig. 2, lanes 1, 3, 4). No such complex was observed when an M1-mutated PEA 3 probe was used (lanes 5, 7). This mutation prevents transactivation by $p68^{c-ets-1}$ (Fig. 1c). Competition experiments showed that the wild-type sequence was the better competitor (Fig. 2, compare lanes 12–15 with 8–11). These results show that $p68^{c-ets-1}$ specifically binds to the PEA 3 motif.

Transcription factors contain activation domains that can stimulate transcription when fused to a heterologous DNA-binding domain. The $p68^{c-ets-1}$ protein fused to the DNA-binding domain of the bacterial repressor LexA (LexA-ets68) activated

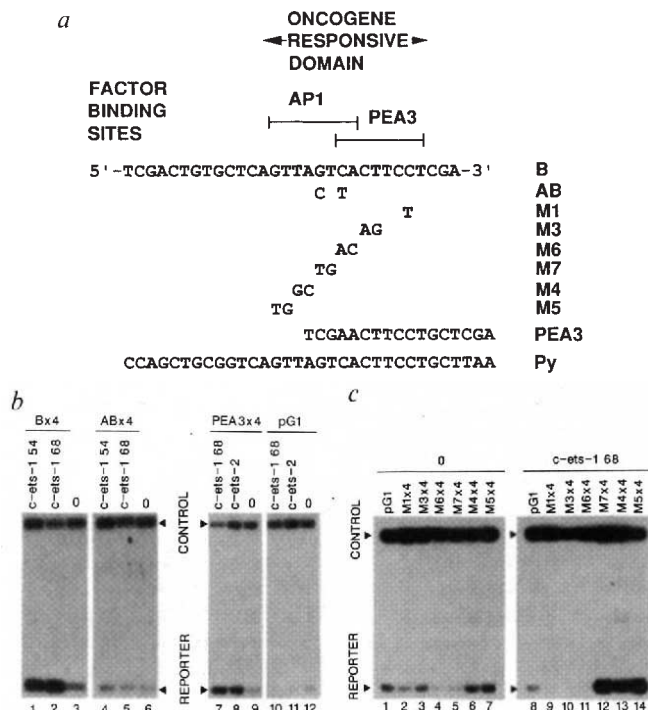


FIG. 1 a, Sequence of transcription elements. Transcriptional activation by the oncogene-responsive domain is measured by the ability of the transcription elements to stimulate transcription from the β -globin gene promoter in the reporter recombinant gene pG1 (ref. 4). The inserts are either four tandem copies of mutants B, AB or M1–M7 (B $\times$ 4, AB $\times$ 4, M1–M7 $\times$ 4) or one copy of the sequences shown. The oncogene-responsive domain of the Py enhancer contains specific binding sites for the transcription factors AP-1 and PEA 3. The extent of the PEA 3 site in the B sequence is deduced from the ability of the wild-type and mutated sequences to specifically mediate PEA 3 binding. The PEA 3 oligonucleotide contains the PEA 3 motif as defined by Martin *et al.*²³. This sequence contains several additional nucleotides from the Py enhancer which compensate functionally for the loss of the C nucleotide that is mutated in the AB oligonucleotide (data not shown, see text). b, Activation of transcription by members of the *ets* gene family. LMTK⁻ mouse fibroblasts were transfected with 1 μ g reporter recombinants (inserts B $\times$ 4, AB $\times$ 4, PEA 3 $\times$ 4 or none (pG1)), 0.5 μ g internal control plasmid (pCB $\times$ 2, ref. 4), and 0.5 μ g expression vectors for $p68^{c-ets-1}$, $p54^{c-ets-1}$ or $p58-64^{c-ets-2}$. c, $p68^{c-ets-1}$ activates transcription through the PEA 3 motif. LMTK⁻ fibroblasts were transfected with 1 μ g reporter recombinants (inserts 4 $\times$ M1, M3, M6, M7, M4 or M5, or none (pG1)), 0.5 μ g internal control (pCB $\times$ 2) and 0.5 μ g c-Ets-1 68 expression vector (lanes 8–14) or the expression vector pSG5 without an insert (lanes 1–7). Reporter and control indicate the corresponding specific S1 nuclease-resistant products.

METHODS. LMTK⁻ cells were transfected with the indicated recombinants by the DEAE dextran and chloroquine technique. The cells were incubated in low-concentration serum for 48 h, then total RNA extracted and analysed by quantitative S1 nuclease mapping for RNAs specifically initiated from the β -globin gene promoter of the reporter recombinant and the conalbumin gene promoter of the internal co-transfected control recombinant⁴. The expression vectors contain the corresponding c-ets complementary DNA sequences in the multiple cloning site of the pSG5 expression vector²⁴.

transcription through the *lexA* operator (Fig. 3, lanes 3, 7). Expression of either LexA alone, or unfused LexA with $p68^{c-ets-1}$, had no effect on transcription (Fig. 3, lanes 1, 2, 5, 6). Coexpression of inactive LexA inhibited LexA-ets68 (Fig. 3, lanes 3, 4, 7, 8), indicating that LexA competes with LexA-ets68 for binding to the operator and thereby inhibits its activity. These results are consistent with the presence of one or more transcriptional activation domains in $p68^{c-ets-1}$.

We previously suggested that there are cooperative interactions between PEA 3 and AP-1 (ref. 4). We tested whether $p68^{c-ets-1}$ could complement AP-1 (c-Fos and c-Jun) to activate transcription from the Py oncogene-responsive domain. Expression in LMTK⁻ cells of limiting amounts of $p68^{c-ets-1}$ or c-Fos and c-Jun proteins led to low levels of activation of the reporter recombinant containing four copies of the B sequence (4- and 20-fold respectively, compared with the effects on the pG1 control reporter; Fig. 4, lanes 5–7 and 1–3). By contrast, coexpression of $p68^{c-ets-1}$ with AP-1 led to a much greater activation (250-fold; Fig. 4, lanes 4 and 8). It is of interest that a monomer B sequence is not detectably activated by either $p68^{c-ets-1}$ or AP-1 expression, and their combined effect is required for efficient activation (results not shown). Both the c-Ets-1 and AP-1 binding sites are required for cooperativity, as $p68^{c-ets-1}$ and AP-1 activate only through their corresponding motifs (ref. 4 and Fig. 1c). There was no complementation when only one of the motifs (PEA 3 or AP-1) was present in the reporter (results not shown). Mutations in part of either motif in the oncogene-responsive domain diminished activation by combined expression of $p68^{c-ets-1}$ and AP-1, but to a lesser extent than would be expected from the effect of the mutation on activation by one of the factors alone (results not shown). These data show that $p68^{c-ets-1}$ can cooperate with AP-1 in transcriptional activation. Expression of $p68^{c-ets-1}$ inhibits basal-level transcription from the oncogene-responsive domain when its own binding site is mutated (0.1-, 0.2- and 0.2-fold for M1, M3 and M6 respectively), but only when the AP-1 site is present

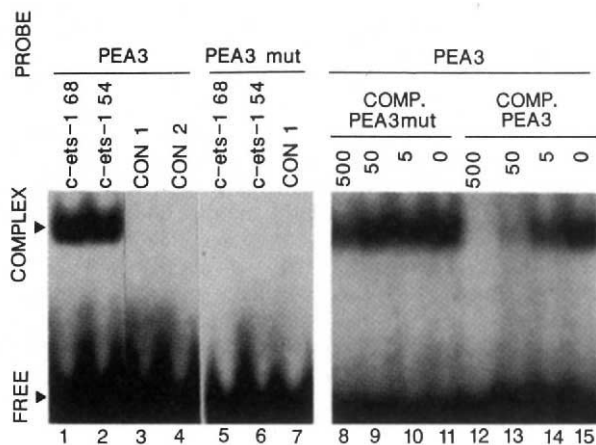


FIG. 2 *In vitro*-synthesized c-Ets-1 specifically binds to the PEA 3 motif. The $p68^{c-ets-1}$ and $p54^{c-ets-1}$ proteins were translated in rabbit reticulocyte lysates (Amersham; as described in the manufacturer's instructions) from RNA transcribed by T7 polymerase from linearized pSG5 expression vectors²⁴ containing cDNAs for c-Ets-1 68 (lanes 1, 5, 8–15), c-Ets-1 54 (lanes 2, 6), or no insert (CON1, lanes 3, 7). Control experiments showed that full-length c-Ets-1 proteins were efficiently synthesized in these lysates. Aliquots (2 μ l) were used for gel-retardation assays with either wild-type (lanes 1–4, 8–15) or mutated (M1 mutation, lanes 5–7) end-labelled PEA 3 probes as described previously⁴. For competition assays (lanes 8–15), the indicated molar excess of cold probe was incubated for 15 min at 0 °C with the *in vitro*-synthesized proteins before the addition of labelled probe. CON1, RNA synthesized from vector without insert; CON2, lysate without added RNA; COMPLEX, specific retarded complex; FREE, excess unretarded probe; COMP, competitor. Similar results were obtained with lysates primed with RNA transcribed by SP6 from different promoter-template combinations (data not shown).

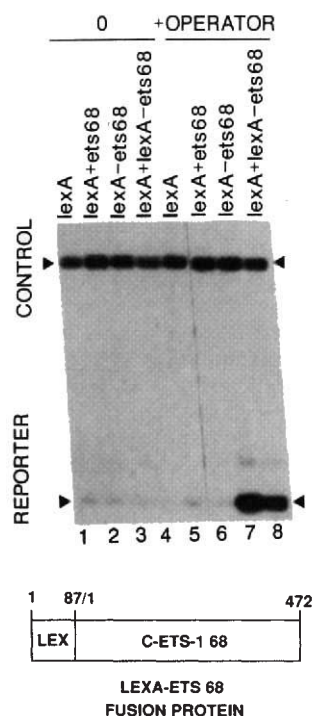


FIG. 3 The p68^{c-ets-1} protein activates transcription when fused to the LexA DNA-binding domain through the *lexA* operator. LMTK⁻ cells were transfected with 1 μ g reporter recombinant containing either the *lexA* operator (lanes 5–8) or no insert (lanes 1–4), 0.5 μ g internal control (pBCB $\times$ 2, ref. 4) and 1 μ g expression vectors that encode either a LexA DNA-binding domain c-Ets-1 68 fusion protein (lanes 3, 7; the expression vector codes for a fusion protein containing LexA amino acids 1–87 and c-Ets-1 amino acids 1–472; bottom), or the LexA DNA-binding domain alone (lanes 1, 5), or separately for the LexA DNA-binding domain and p68^{c-ets-1} (lanes 2, 6), or separately for the LexA DNA-binding domain and the LexA-Ets fusion protein (lanes 4, 8).

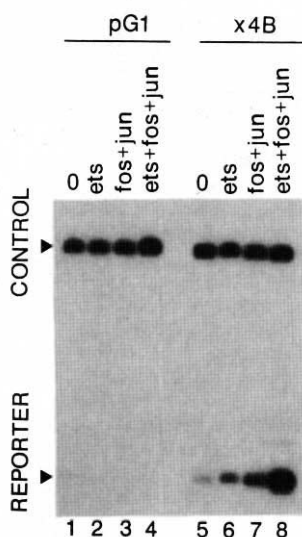


FIG. 4 Complementation between p68^{c-ets-1} and c-Fos and c-Jun (AP-1) for transcriptional activation through the oncogene-responsive domain. LMTK⁻ fibroblasts were transfected in low serum concentrations (0.05% FCS) with 1 μ g reporter recombinants containing either four copies of the B mutant (lanes 5–8) or no (lanes 1–4) insert, 0.5 μ g internal control (pBCB $\times$ 2) and 0.1 μ g expression vectors for c-Ets-1 68, c-Fos or c-Jun⁴ as indicated. Total RNA was analysed by quantitative S1 nuclease analysis. Reporter and control indicate the corresponding specific bands.

(see pG1, Fig. 1c, lanes 1 and 8). Similar results were obtained with a monomeric oncogene-responsive domain (data not shown). These observations could be explained by a squelching-type mechanism, in which p68^{c-ets-1} specifically binds to AP-1; but other mechanisms cannot be excluded at present.

Expression of p54^{c-ets-1}, which differs from p68^{c-ets-1} at its N terminus¹⁰, activated transcription through the PEA 3 motif (Fig. 1b, lanes 1 and 4, and results not shown). The p54^{c-ets-1} protein specifically bound to the PEA 3 motif (Fig. 2, lanes 2, 6, and data not shown). Therefore, the sequence differences between p68 and p54 do not significantly affect their ability to activate transcription in the systems tested.

Expression of p58-64^{c-ets-2}, another member of the *ets* gene family¹¹, activated transcription through the PEA 3 motif to a similar extent as expression of p68^{c-ets-1} (Fig. 1b, lanes 8, 9 and 11, 12). These results indicate that the domains conserved between c-Ets-1 and c-Ets-2 are required for transcriptional activation.

The c-Ets proteins have DNA-binding and transcriptional activation domains. It seems likely that the DNA-binding domain is located in the C-terminal part of the protein that is basic, highly conserved between different *ets* gene family members^{10–15} and involved in nonspecific DNA-interactions^{16–20}. DNA-binding domains are highly conserved in other families of transcription factors^{3,21}. Preliminary evidence indicates that the N terminus contains an activation domain (data not shown). There is no obvious homology between the *ets* family and other transcriptional activators, indicating that this is a new family of transcription factors. The oncogene-responsive domain of the Py enhancer and collagenase promoter are functionally equivalent to the type II proto-enhancer, where the association of two different motifs, which are weakly active on their own, generates a proto-enhancer, the building block of enhancers²². The oncogene-responsive domain represents a particular form of the type II proto-enhancer in that its activity is regulated by mitogens (and many oncogenes) and it contains the motifs for three oncoprotein transcription factors. It is of interest that associated PEA 3 and AP-1 motifs are found in several oncogene-responsive promoters of the genes for stromelysin, interleukin-2, Fos and JE (see ref. 4 for discussion). The importance of *ets* and *fos* and *jun* in mediating transcriptional activation and transformation by non-nuclear oncogenes remains to be established. □

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The candidate Wilms' tumour gene is involved in genitourinary development

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WILMS' tumour is an embryonic kidney tumour thought to arise through aberrant mesenchymal stem cell differentiation¹ and to result from loss of function of a 'tumour suppressor' gene(s)². Both sporadic and syndrome-associated Wilms' tumours are accompanied by an increased frequency of abnormalities of the urinary tract and genitalia³. Deletional analysis of individuals with the WAGR syndrome⁴⁻⁸ (for, Wilms' tumour, aniridia, genitourinary abnormalities and mental retardation) showed that a Wilms' tumour gene lies at chromosomal position 11p13. This led to the isolation of a candidate Wilms' tumour gene^{9,10}, encoding a zinc-finger protein which is likely to be a transcription factor. To gain insight into the role of this candidate gene in normal development and tumorigenesis, we have now performed *in situ* messenger RNA hybridization on sections of human embryos and Wilms' tumours. The candidate Wilms' tumour gene is expressed specifically in the condensed mesenchyme, renal vesicle and glomerular epithelium of the developing kidney, in the related mesonephric glomeruli and in cells approximating these structures in tumours. The other main sites of expression are the genital ridge, fetal gonad and mesothelium. These data suggest that (1) this candidate is indeed a Wilms' tumour gene, (2) the associated genital abnormalities are pleiotropic effects of mutation in the Wilms' tumour gene itself, in support of recent genetic analysis¹¹, and (3) this gene has a specific role in kidney development and a wider role in mesenchymal-epithelial transitions.

We have investigated the expression of the candidate Wilms' tumour gene by northern blot analysis and by *in situ* hybridization. For this we used the complementary DNA probe *WT2-1*, known to detect a transcript of ~3 kilobases (kb) in fetal kidney and spleen⁹. Northern analysis (Fig. 1) showed that this probe detected a 3.2-kb transcript not only in fetal kidney and spleen but also in fetal testis and ovary, with an additional 2.7-kb transcript detectable in fetal testis. There was very weak expression in fetal brain. No expression was detected in fetal heart, skin, adrenal, stomach, liver, eye or muscle.

In situ mRNA hybridization was first carried out on 18-week-gestation human kidney, chosen because it includes all stages of nephron development from uninduced blast cells at the periphery to functioning nephrons towards the centre. This full range of stages is seen because the process of kidney development, involving a reciprocal inductive interaction between the ureteric bud and the metanephric blastema, is continuous from 6-34-weeks gestation^{12,13}. If the candidate gene (*WT2-1*) is involved in the initiation of Wilms' tumour, then, to explain tumour histogenesis we should expect to find it expressed in the multipotent blast cells of the kidney.

The *WT2-1* gene was expressed in a very specific manner within the developing nephron (Fig. 2a). Expression was weak

in the condensing blastemal cells, stronger in the renal vesicle—an epithelial structure arising from the condensate—and then restricted to the maturing renal corpuscle. Here, expression was particularly strong in the developing podocyte cells of the glomerular epithelium, but, as the corpuscle matured, expression declined. By contrast, no expression could be seen in the remainder of the nephron (the proximal and distal tubules and loops of Henlé) or in the derivatives of the ureteric bud (the ureter and collecting tubules). A similar but much less intense pattern of glomerular labelling was seen in normal kidney adjacent to a Wilms' tumour removed from a 10-month-old male (data not shown).

To investigate whether the gene has a role in earlier development, we examined sections of 6-7-week human embryos by *in situ* mRNA hybridization (Fig. 2b). Here, *WT2-1* expression in the metanephric blastema matched that in the 18-week kidney; it was very strong in the condensing blast cells, but absent in the uninduced mesenchyme and ureteric bud branches. The *WT2-1* gene was also expressed in the glomerular epithelium of the related mesonephros and in the developing gonad, mainly over the germinal epithelium and the sex cords, but not over those germ cells that could be identified morphologically. Of the remaining tissues that could be seen clearly, expression was restricted to the mesothelial lining of the coelomic cavity and its contents, with some expression in the somatopleuric mesoderm immediately underlying this layer dorsolaterally.

If *WT2-1* plays a primary role in Wilms' tumour, we would expect mutations affecting this gene to be present in a significant number of tumours, although perhaps not in all, as more than one locus has been implicated in the disease¹⁴⁻¹⁷. We analysed 13 tumours by northern blot (Fig. 3) and found that each expressed the 3.2-kb transcript. This result is not unexpected in the light of retinoblastoma observations, where most fresh

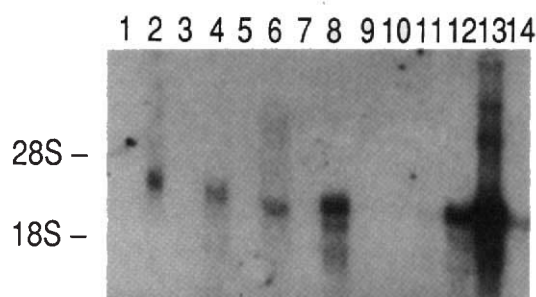


FIG. 1 Northern blot analysis of *WT2-1* expression in human fetal tissues. Lanes: 1, adrenal; 2, kidney; 3, heart; 4, ovary; 5, skin; 6, spleen; 7, stomach; 8, testis; 9, eye; 10, liver; 11, brain; 12, HEK1; 13, kidney; 14, 293 cells. Lanes 1-7, total RNA; and 8-14, poly(A)⁺ RNA. HEK1 is a primary culture of human fetal kidney cells. 293 is an adenovirus-transformed human embryonic kidney cell line (ATCC number: CRL 1573). A 3.2-kb transcript is present in fetal kidney, spleen, testis, ovary and brain, with an additional 2.7-kb transcript seen in testis when poly(A)⁺ RNA is used. In this experiment the RNAs ran on a slant, but we have confirmed in independent analyses that the 3.2-kb transcript is the same size in all positive tissues. Size markers shown (left).

METHODS. Human fetal tissues were obtained at autopsy of 10-19-week gestation fetuses. RNA was prepared from frozen tissues by the guanidine isothiocyanate/cesium chloride gradient method²⁴. Total RNA (20 µg) or poly(A)⁺ RNA (10 µg) was loaded, electrophoresed through 1% agarose-formaldehyde gels and transferred to Hybond-N, as described²⁵. Filters were probed with *WT2-1*, a 1.5-kb *EcoRI*-*PstI* fragment of cDNA 2-1. This was isolated from a human fetal kidney cDNA library by screening with the conserved single copy probe J803p4, which maps to the Wilms' tumour locus at 11p13 (ref. 9). High-specific-activity ³²P-labelled DNA probes were prepared for northern blot hybridization using a Random Prime kit according to manufacturers' instructions (Boehringer-Mannheim). Hybridization was at 68 °C in 7% SDS, 0.5 M sodium hydrogen phosphate, 1 mM EDTA. Filters were washed at 68 °C in 2×SSC, 0.1% SDS and exposed to Kodak XAR film for 18 h. Loadings were standardized by reprobing with actin (data not shown).

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FIG. 2 *a-f*, Expression pattern of *WT2-1* in an 18-week gestation human fetal kidney as revealed by *in situ* mRNA hybridization. All stages of nephrogenesis (see refs 12 and 13 for reviews) are shown. The active nephrogenic zone is most peripheral. *a*, Low power view of longitudinal section of kidney, hybridized with antisense probe. Labelling is restricted to the subcortical condensed blastema, renal vesicle and podocyte layer of the renal corpuscle. More mature corpuscles (arrow) lie farthest from the cortex and label less strongly. Bar, 1 mm. *c*, High power view of *a* showing labelling confined to the presumptive podocyte layers (arrow) of the S-shaped body (right) and immature glomerulus (left). Bar, 200 μ m. *b* and *d*, Dark-field view of *a* and *c*, respectively. *e*, High-power view of *a* showing uniformly labelled renal vesicle (arrow) adjacent to its inducing ureteric bud ampulla (hollow arrow), which does not label. Bar, 100 μ m. *f*, Similar structure to *e* from adjacent section of same kidney hybridized to sense (control) probe. RV, renal vesicle; U, ureteric bud ampulla. *g-i*, Expression pattern of *WT2-1* in human embryos, as revealed by *in situ* mRNA hybridization. *g*, Transverse section through upper thoracic region of a 43-day human embryo, hybridized with antisense riboprobe for *WT2-1*. Bright field reveals structure only. Lu, lung bud; H, heart; Li, liver. Bar, 0.5 mm. *h*, Same section viewed under dark field illumination to reveal silver grains showing sites of binding of *WT2-1*. Labelling of the liver parenchyma is nonspecific as it also occurs with the control (sense) probe. *i*, Higher-power view of *h* showing labelling of pleural, peritoneal and pericardial layers. Pe, pericardium; PP, parietal peritoneum; VP, visceral peritoneum; P indicates both pleural layers. Bar, 200 μ m. *j*, Adjacent section to *g* labelled with sense *WT2-1* riboprobe. Liver binding is nonspecific. *k*, Transverse section through lower abdominal region of a 49-day gestation human embryo, labelled with antisense *WT2-1* riboprobe. Heavy labelling is seen over the gonadal ridge (G), condensed metanephric blastema (C), glomerular structures of the mesonephros (MG) and the epithelial layer lining the coelomic cavity. The mesonephric duct (MD), ureteric bud branches (U) and uncondensed metanephric mesenchyme (M) are unlabelled. An adjacent section hybridized with the sense probe showed no labelling (data not shown). Bar, 200 μ m. *l*, Same section as *k* viewed under dark-field illumination.

METHODS. Fetal kidneys were obtained at autopsy and embryos were obtained following legal termination of pregnancy. Specimens were fixed in 4% paraformaldehyde and wax-embedded. Sections (7 μ m) were mounted onto TESPA-coated slides. *In situ* hybridization was performed as described²⁶, including hybridization at 55 °C in 50% formamide, high stringency washes at 65 °C in 50% formamide and RNase A treatment. Antisense and sense (control) riboprobes for the *WT2-1* gene were prepared by [³⁵S] UTP incorporation; 1.2×10^6 d.p.m. were applied to each section. In all experiments, the sense probe was routinely included and gave no labelling above background.

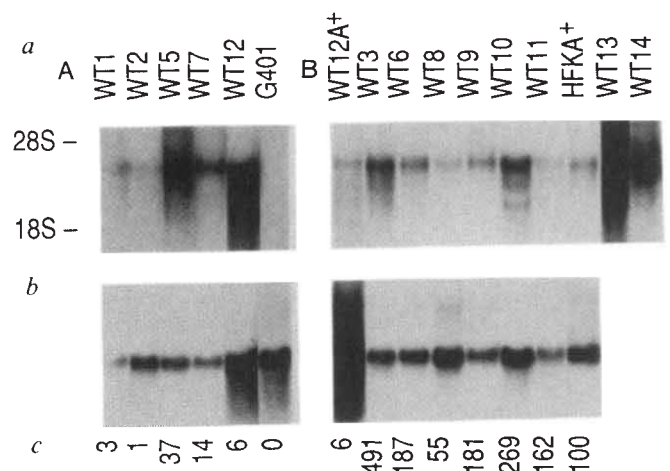
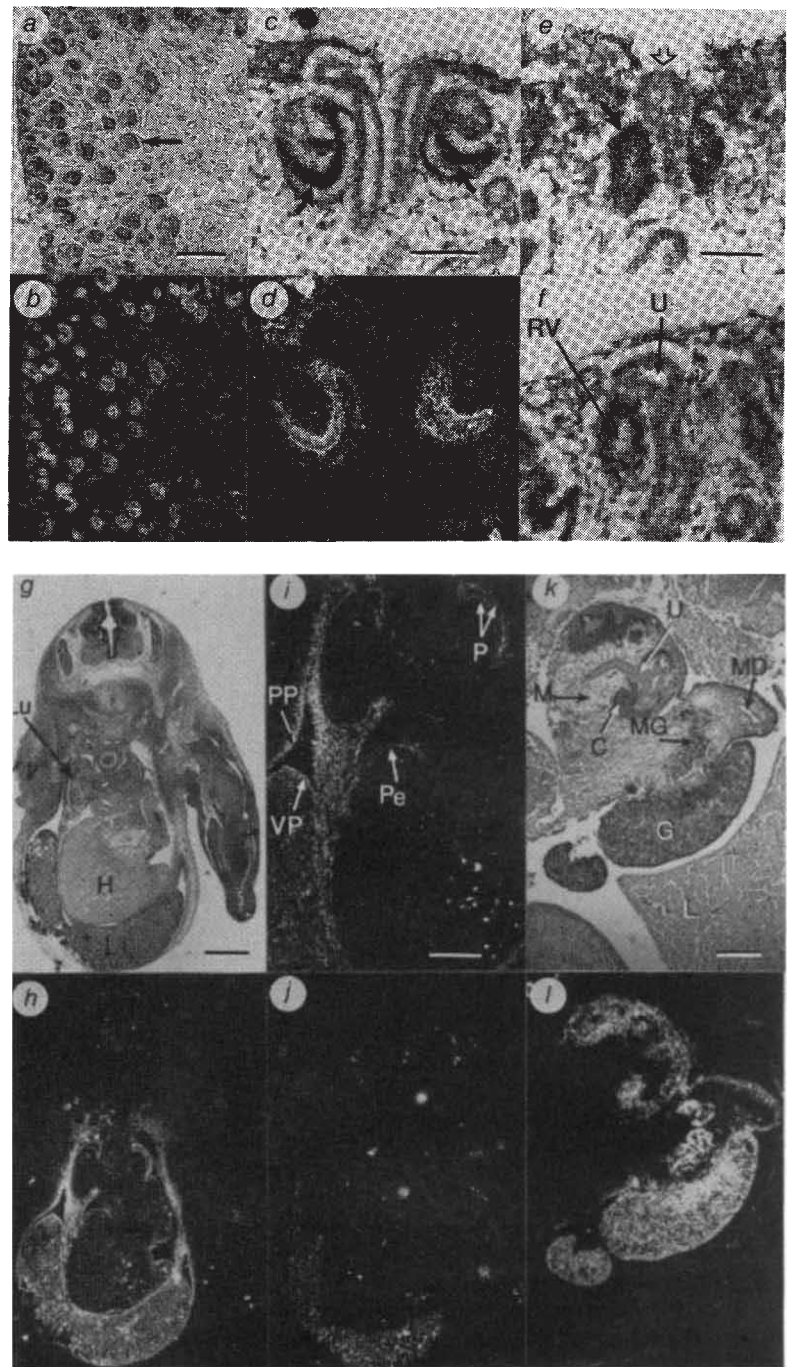


FIG. 3 Northern blot showing variation in level of *WT2-1* expression in Wilms' tumours. The same filters were hybridized to *WT2-1* (*a*) or glyceraldehyde phosphate dehydrogenase²⁷ (*b*) (autoradiographs were exposed for 48 h (*A*) and 5 h (*B*)). WT13 and 14 were run separately and exposed for 36 h. G401, Wilms' tumour cell line. Autoradiographs were scanned densitometrically²⁸ and the level of *WT2-1* expression relative to that of human fetal kidney (100%) is shown in *c*, using GAPDH to standardize loadings. Methods as in Fig. 1, but filters were washed in $0.5 \times \text{SSC}$.

tumour samples express a full-length transcript of the retinoblastoma gene¹⁸, mutations only being revealed by methods detecting point mutations or small rearrangements¹⁹. We did, however, find an almost 500-fold variation in *WT2-1* expression, an observation that prompted us to try to correlate retrospectively expression with tumour histology. We found that tumours that were predominantly blastemal or showed epithelial differentiation expressed high levels of *WT2-1*, but tumours with stromal predominance had low levels. This correlation was confirmed directly by *in situ* mRNA hybridization analysis of seven Wilms' tumours (Fig. 4); highest levels of expression were found in tubular structures at a stage immunohistochemically equivalent to the early renal vesicle in normal development, while blastemal cells showed lower levels of expression but were clearly positive relative to the nonexpressing stromal cells. Thus, *WT2-1* expression is found only in the malignant counterparts of cell types that express the gene during normal kidney development.

Our results, taken together with the now precisely known chromosomal localization of the gene and its structural homologies^{9,10}, strengthen the case for *WT2-1* being a Wilms' tumour gene.

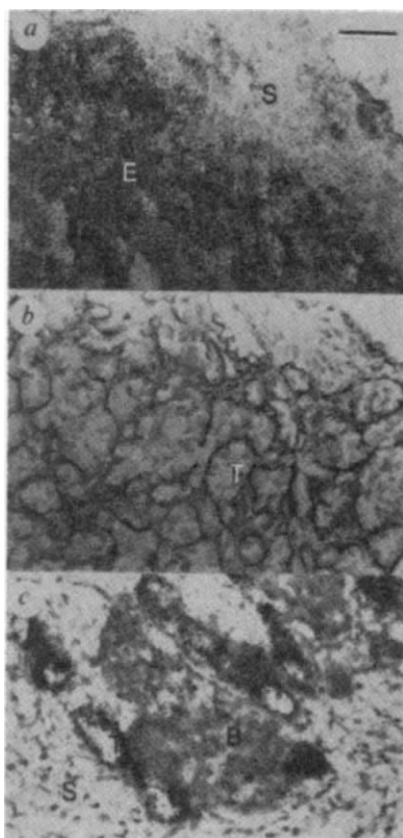


FIG. 4 Expression pattern of *WT2-1* in Wilms' tumours WT13 and 14 as shown by *in situ* mRNA hybridization. *a*, WT13 hybridized with antisense probe. There is heavy labelling over areas of epithelial differentiation (E) but none over stromal areas (S). Bar, 100 μ m. *b*, Similar area in nearby section of same tumour, labelled immunocytochemically with antibodies to laminin, showing polarized basement membranes of tubular structures. Antibodies to collagen IV gave a similar result. These tubules were negative for cytokeratins 8 and 18 on an adjacent section labelled with CAM 5.2, suggesting that they are at a stage equivalent to the early renal vesicle in normal development^{29,30} and not the mature tubule. *c*, WT14 hybridized with antisense probe. Labelling is seen over blastemal cells (B) and tubular structures (T) but not over the stroma (S). Similar patterns of expression were seen in five other Wilms' tumours (data not shown).

METHODS. Tumours were obtained fresh at the time of nephrectomy. Tissue fixation and *in situ* hybridization were performed as described in Fig. 2. Immunohistochemical staining was performed using polyclonal rabbit anti-laminin antibodies (Eurodiagnostics) at a 1 in 50 dilution by the peroxidase-antiperoxidase method, as described³¹.

The specific expression pattern of *WT2-1* contrasts with the behaviour of the retinoblastoma gene—a putative cell-cycle regulator—which is ubiquitously expressed, but gives rise through unknown mechanisms to tumours that are tissue-restricted²⁰. As to the origins of Wilms' tumour, our observations lead us to the following hypothesis. During normal kidney development, the *WT2-1* gene product acts as a transcriptional regulator to switch off genes involved in maintaining cell proliferation and/or switch on genes involved in blastemal differentiation. In tumours, we suggest that the gene retains its responsiveness to its upstream regulators in the proliferative response pathway, so that it is transcribed in the appropriate cell types, but that mutation alters the ability of its product to regulate downstream target gene(s), so leading to uncontrolled proliferation with aberrant differentiation and eventual tumour formation.

The observations presented here also cast light on other aspects of phenomena associated with normal and abnormal kidney development. The fact that the gene is expressed in both the podocytes and the more primitive mesonephros argues for it playing an important part in the development of the specialized filtration function of the kidney, a role that may have evolved early in vertebrate evolution. More intriguing, perhaps, is the observation that the gene is expressed in three tissues undergoing the rare transition from mesenchyme to epithelium: the differentiation of the metanephric mesenchyme to nephrons, the formation of the mesothelium from the mesenchyme lining the coelom and the production of sex cords from the mesenchyme of the primitive gonad. It will be interesting to investigate whether tumours from these tissues express the *WT2-1* gene.

The expression of *WT2-1* in the developing gonad gives a molecular basis for several phenomena seen in individuals with Wilms' tumour. First, the genital abnormalities seen as part of the WAGR syndrome and associated with sporadic Wilms' tumour could be pleiotropic effects of mutation in the Wilms' tumour gene. Second, both the pseudohermaphroditism and nephropathy seen as part of the Wilms' tumour-associated Drash syndrome²¹ could be accounted for by disruption of a single gene. Finally, the lack of inheritance of Wilms' tumour-predisposing mutations at the 11p13 locus^{14,15} could be due to such mutations causing infertility. This speculation arises from our observation of *WT2-1* expression in non-germ-cell components of the fetal gonad, from which develop the epithelial components of both testis and ovary. In abnormalities of gonadal epithelial development, both gametogenesis and the hormone-dependent development of the external genitalia may be faulty²². Thus, both infertility and anatomical abnormalities of the genitalia could result from mutation or reduced dosage of this gene, perhaps in a manner analogous to that described for testis determination.²³ □

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TABLE 1 Ablation of all gonadal cells except Z1.ppp or Z4.aaa in *lin-12(d)* mutants

<i>lin-12</i> genotype*	<i>n</i> [†]	Fate [‡] of Z1.ppp or Z4.aaa
+	5/5 [§]	AC
<i>n379</i>	3/3	VU
<i>n349</i>	4/4	VU
<i>n676</i>	4/4	VU
<i>n952</i>	7/7	VU
<i>n137</i>	8/8	VU

Ablations were performed as described in ref. 3.

* Complete genotypes: *n379* is *lin-12(n379); him-5(e1467)*. *n952* is *unc-32(e189) lin-12(n952)*. *n137* is *lin-12(n137); him-5(e1467)*.

[†] *n*, Number of animals that exhibited the cell fate indicated divided by the total number of animals examined.

[‡] AC, anchor cell; VU, ventral uterine precursor cell. Criteria for scoring cell fates were as described³.

[§] Data from refs 1 and 3.

^{||} Data from ref. 3.

contrast, in all five *lin-12(d)* mutants examined, an isolated Z1.ppp or Z4.aaa expresses the VU fate (Table 1)³. These results suggest that *lin-12(d)* protein is active in the absence of the 'AC-to-VU' signal, is capable of autocrine activation by the signal produced by the isolated cell, or is capable of activation by low levels of the signal present in cellular debris. These results imply not only a functional difference between *lin-12(d)* and *lin-12(+)* proteins, but also a functional similarity among the *lin-12(d)* proteins, because all *lin-12(d)* alleles behave the same way by this laser ablation criterion.

We next analysed the behaviour of *lin-12(d)* mutations in heteroallelic combinations with the *lin-12(n302 n653)* allele (Table 2), which is associated with two point mutations. One, *n302*, is a *lin-12(d)* mutation in the putative extracellular domain; the other, *n653*, is in the putative intracellular domain (see Fig. 1 and Table 2). The phenotype of *lin-12(n302 n653)* homozygotes at 25 °C suggests that *lin-12* activity is strongly reduced: *lin-12(n302 n653)* homozygotes have two ACs (Table 2) as do *lin-12(0)* hermaphrodites. As expected for an allele that reduces *lin-12* activity, *lin-12(n302 n653)* is completely recessive to *lin-12(+)* (Table 2), even in hermaphrodites of *lin-12* genotype *lin-12(n302 n653)/lin-12(n302 n653)*; *mnDp37[lin-*

12(+)] (data not shown). Table 2, however, demonstrates that at 25 °C *lin-12(n302 n653)* does not behave as a simple reduced-activity allele in heteroallelic combinations, that is, it does not have *lin-12* activity intermediate between that of *lin-12(+)* and *lin-12(0)*. Instead, in combination with *lin-12(d)* alleles, *lin-12(n302 n653)* seems to have greater activity than a *lin-12(+)* allele. This result suggests that a *lin-12(d)* protein can transactivate or stabilize the protein encoded by *lin-12(n302 n653)*. In addition, the degree of proposed transactivation seems to be *lin-12(d)* allele-specific: although *lin-12(n952)* seems to elevate *lin-12* activity more than *lin-12(n302)* in gene-dosage studies with *lin-12(+)* and *lin-12(0)* alleles, fewer *lin-12(n952)/lin-12(n302 n653)* hermaphrodites than *lin-12(n302)/lin-12(n302 n653)* hermaphrodites display the gain-of-function phenotype (Table 2). This apparent allele-specificity supports the interpretation of a direct association between proteins encoded by *lin-12(d)* and *lin-12(n302 n653)* and, by extension, between those encoded by *lin-12(d)*.

We can perhaps also explain how *lin-12(+)* alleles augment *lin-12(d)* activity, by proposing that a *lin-12(d)* protein can transactivate a *lin-12(+)* protein. But it remains possible that the *lin-12(+)* allele contributes a basal level of *lin-12* activity that can augment *lin-12(d)* activity.

Finally, we postulate that the *lin-12(+)* protein also self-associates to form dimers or multimers, leading to activation. Genetic evidence for self-association of the *Drosophila Notch* protein^{11,12}, which is similar to the *lin-12* protein in overall predicted structure^{6,13,14}, has recently been supported by direct biochemical evidence of dimerization¹⁵. In this context, it is noteworthy that the region of the *lin-12* protein affected in *lin-12(d)* mutants includes a pair of cysteines also present at a corresponding position in Notch, although the extracellular cysteine-poor regions of these two proteins do not display any other notable amino-acid similarity⁶. The two cysteines are also present in a corresponding position in the predicted protein products of other members of the *lin-12/Notch* family, including another *C. elegans* gene, *glp-1* (ref. 16), and genes from *Xenopus* (C. Coffman, C. Kintner & W. Harris, personal communication) and humans (L. Ellisen & J. Sklar, personal communication). These two cysteines—the only two in that region of the protein—may be responsible for the sulphhydryl-mediated dimerization of Notch¹⁵.

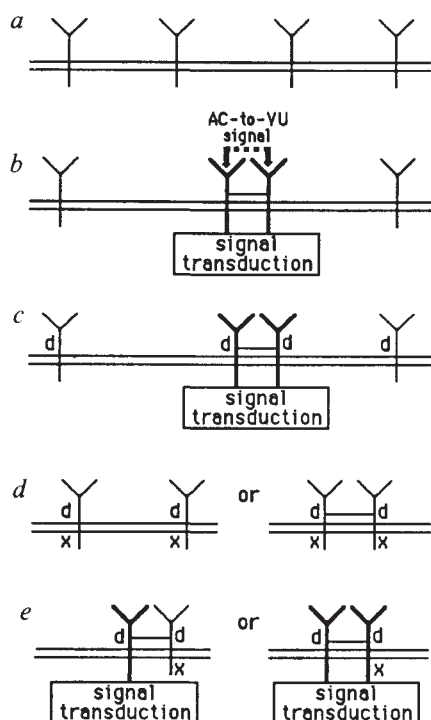


FIG. 2 A model for how dimerization leads to activation of the *lin-12* protein. *a*, In wild type, *lin-12* is a receptor that is inactive in the absence of its ligand. *b*, In wild type, the ligand ('AC-VU' signal) promotes dimerization, leading to signal transduction by means of the intracellular domain. Ligand-binding to receptor monomer may cause a conformational change that results in or stabilizes dimerization; alternatively, the ligand may have a more direct role in a cross-linking mechanism. *c*, In a *lin-12(d)* homozygote, dimerization and signal transduction are ligand-independent. The *lin-12(d)* mutations might result in ligand-independent activation of the *lin-12* protein by inactivating a negative regulatory domain or by causing changes in the higher-order structure. *d*, In a *lin-12(n302 n653)* homozygote, the *n653* mutation either prevents homodimer formation or inactivates *lin-12*. *e*, In a *lin-12(d)/lin-12(n302 n653)* heterozygote, heterodimers can form and are active.

TABLE 2 Heteroallelic combinations between *lin-12(n302 n653)* and *lin-12(d)* alleles

<i>lin-12</i> genotype*	Egl/total†	Percentage of Egl
+/ <i>n302 n653</i>	0/203‡	0
<i>n379/n302 n653</i>	143/237	40
<i>n379/+</i>	38/357	11
<i>n379/lin-12(0)</i>	14/252	6
<i>n302/n302 n653</i>	226/296	76
<i>n302/+</i>	101/204	50
<i>n302/lin-12(0)</i>	36/184	20
<i>n952/n302 n653</i>	156/245	64
<i>n952/+</i>	186/299	62
<i>n952/lin-12(0)</i>	63/199	32
<i>n676/n302 n653</i>	202/214§	94
<i>n676/+</i>	183/260	70
<i>n676/lin-12(0)</i>	154/425	36
<i>n137/n302 n653</i>	112/112	100
<i>n137/+</i>	256/289	89
<i>n137/lin-12(0)</i>	88/190	46

All experiments were performed at 25 °C, as the behaviour of *lin-12(n302 n653)* is temperature-dependent (G.S. and I.G., unpublished observations). If the *lin-12(d)* heterozygote and hemizygote data presented here are compared with the data at 20 °C (ref. 4), the penetrance of *lin-12(n952)* and *lin-12(n137)* is lowered and that of *lin-12(n379)* is elevated. The *lin-12(n302)* hermaphrodites are egg-laying defective (Egl), a direct consequence of the transformation of the presumptive AC into a VU⁴. Because the penetrance of the Egl defect is incomplete in *lin-12(d)/lin-12(0)* and *lin-12(d)/lin-12(+)*, wild-type (non-Egl) revertants containing alleles that lower the activity of *lin-12(n302)* may be obtained. The most common intragenic revertant class obtained after ethyl methanesulphonate mutagenesis comprises *lin-12(0)* alleles⁴; *lin-12(n302 n653)* is one of a heterogeneous class of non-null revertant alleles (I.G., unpublished observations). At 25 °C, in the dissecting microscope, *lin-12(n302 n653)* homozygotes all have 2AC (52/52), so it resembles a *lin-12(0)* allele for the AC/VU decision. But *lin-12(n302 n653)* seems to retain some *lin-12* activity: only 52% (71/136) have the characteristic *lin-12(0)* ventral protrusion; the remainder have a smaller vulval protrusion. The *lin-12(n302 n653)/lin-12(0)* hermaphrodites resemble *lin-12(n302 n653)* homozygotes.

* We represent here a marker linked to *lin-12* as 'mar'. Total non-Mar non-Unc self-progeny were counted from heterozygotes of genotype *mar lin-12(d)/unc-36(e251) lin-12(n302 n653)*; *him-5(el467)/+*, *mar lin-12(d)/unc-36(e251)*; *him-5(el467)/+*, or *mar lin-12(d)/unc-36(e251) lin-12(n137 e2034::Tol)*; *him-5(el467)/+*. For *n379*, *n302* and *n676*, *mar* is *dpy-17(e164)*; for *n952*, *mar* is *unc-32(e189)*; for *n137*, *mar* is *dpy-19(e1259)*. *lin-12(n137 e2034::Tol)* is a *lin-12(0)* mutation.

† Egl, number of hermaphrodites that display an Egl defect associated with elevated *lin-12* activity (the AC/VU cell fate transformation)⁴. To verify that *lin-12(d)/lin-12(n302 n653)* hermaphrodites displayed this transformation, we examined the progeny of *unc-36(e251) lin-12(n302 n653)/dpy-17(e164) lin-12(n379)*; *him-5(el467)/+* hermaphrodites in the L3 stage for the presence of an AC: 16/37 (43%) hermaphrodites did not have an AC, and 6/6 of these hermaphrodites were Egl; 21/37 hermaphrodites had one AC, and 7/7 of these hermaphrodites were non-Egl.

‡ 2/203 *unc-36(e251) lin-12(n302 n653)/++* hermaphrodites had an abnormal vulva that affected egg-laying, but the abnormality was not of the sort associated with elevated *lin-12* activity.

§ The *lin-12(n676)* homozygotes, heterozygotes and hemizygotes display the AC to VU transformation but rarely display a 'multivulva' (Muv) phenotype, which is caused by transformation of the vulval precursor cell fates⁴. The 'strong' *lin-12(d)* mutants *lin-12(n950)*, *lin-12(n137)* and *lin-12(n177)* display a Muv phenotype as homozygotes, heterozygotes and hemizygotes; *lin-12(n952)* mutants display a Muv phenotype only as homozygotes. It is therefore striking that most *lin-12(n676)/lin-12(n302 n653)* hermaphrodites displayed a Muv phenotype; the lineages of the vulval precursor cells were not determined.

We incorporate the genetic and molecular findings reported here into our receptor model for *lin-12* function³ (Fig. 2). According to this model, *lin-12* encodes a receptor that becomes activated on binding of its ligand. The ligand activates the *lin-12* protein by promoting its self-association, and on activation, the

intracellular domain induces signal transduction. This model is plausible in view of what is known about biochemically defined receptors. In many cases, the association state of receptors in the membrane controls their activity, and association state is in turn controlled by ligand-binding¹⁷⁻²⁰. Although the available data are consistent with the model proposed here, other interpretations are possible. Thus, a future challenge will be to obtain biochemical evidence to confirm or disprove this model. □

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RNA-polymerase specificity of transcription of *Arabidopsis* U snRNA genes determined by promoter element spacing

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ALTHOUGH most eukaryotic genes known to be transcribed by RNA polymerase III have intragenic promoter elements, some are similar to genes transcribed by RNA polymerase II in that they have upstream promoters (reviewed in refs 1-4). Transcription of the vertebrate U6 and 7SK RNA genes by RNA polymerase III depends exclusively upon upstream signals, some of which are indistinguishable from the elements essential for polymerase II-specific genes⁵⁻¹¹. In the plant *Arabidopsis thaliana* the promoter elements for the U6 and U2 small nuclear RNA genes, transcribed by RNA polymerases III and II respectively, are identical, comprising a -30 TATA box and an upstream element specific for small nuclear RNA genes^{12,13}. The distance between these elements differs, however. Here we report evidence that this separation is crucial in determining whether the genes are transcribed by polymerase II or III.

Figure 1 illustrates the organization of the upstream promoter elements for the plant U small nuclear (sn) RNA genes transcribed by RNA polymerases II and III (pol II and pol III). In pol II-specific genes the upstream sequence element (USE) and the TATA-like box are centred roughly four DNA helical turns apart, whereas in pol III U6 genes the spacing is about one helical turn less. Hybrid U snRNA genes were prepared to

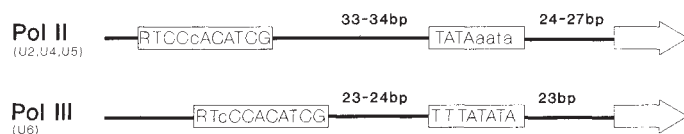
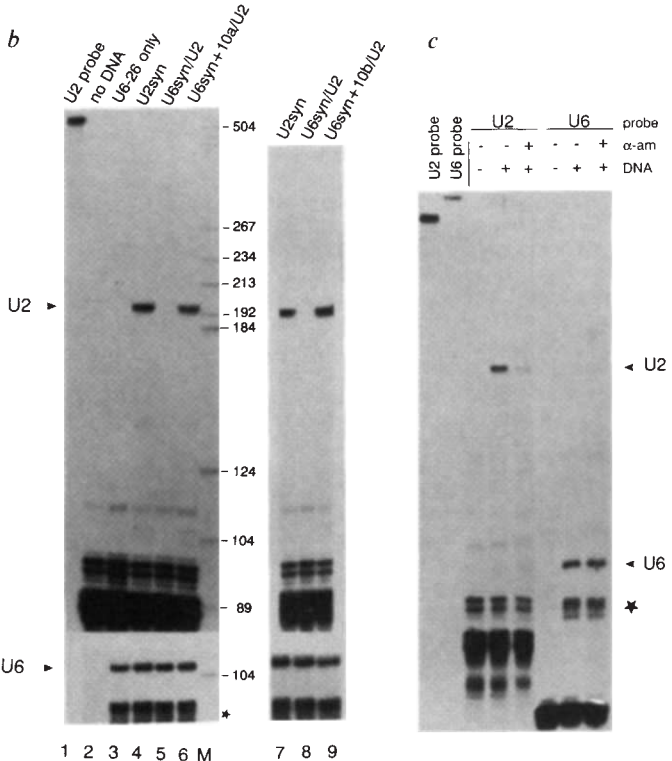
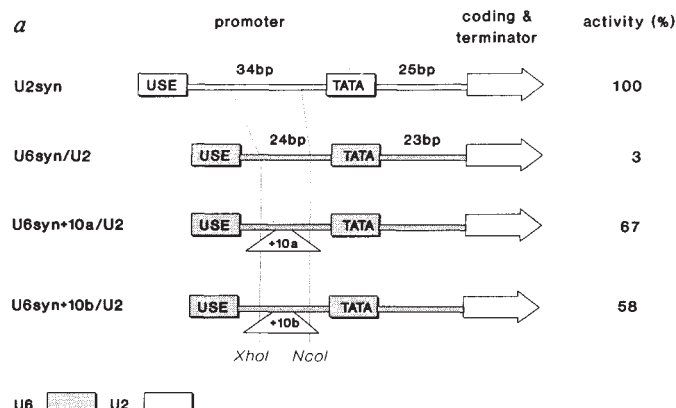


FIG. 1 Conserved upstream elements of pol II- and pol III-transcribed U snRNA genes of *Arabidopsis*. The pol II consensus is based on the sequences of six U2 (ref. 18), one U4 (our unpublished results) and one U5 gene¹⁵. U1 RNA genes isolated from bean, soybean and tomato contain the same conserved elements at similar positions^{21,22}. The pol III consensus is based on the sequences of three active U6 genes (F. W. and W. F., manuscript submitted). Elements found in both pol II and pol III promoters are boxed. Nucleotides not conserved in all genes are in lower case. The coding region is indicated by an arrow. For both U2 and U6 genes of *Arabidopsis* the USE and TATA seem to be the only essential promoter elements and the TATA elements of U2 and U6 genes are exchangeable. The evidence that U2, U4 and U5 genes are pol II-specific is based on the presence of m³G caps in the RNA products and on the sensitivity of transcription in protoplasts to α -amanitin. Transcription of the U6 genes is resistant to α -amanitin and terminates within stretches of T residues, which function as pol III terminators^{12,13}.

examine whether a pol III promoter can be converted into a pol II promoter and vice versa, by changing the distance between the elements. In the construct U6syn/U2 (Fig. 2a) the promoter of the model U6syn gene was fused with an *Arabidopsis* U2 gene lacking its own promoter¹². The resulting gene contains a 70-base-pair (bp) U6syn promoter followed by 196 bp of the coding and 264 bp of the downstream noncoding U2 gene sequence. Transfection of the hybrid U6syn/U2 gene into protoplasts of *Nicotiana plumbaginifolia* resulted in accumulation of only trace levels of U2 RNA (Fig. 2b). This observation is not unexpected. If RNA synthesis from this construct were indeed

initiated by pol III, transcription would probably terminate in the coding region of the U2 gene where stretches of T residues, which act as pol III terminators¹⁴, are present. The accumulation of mature U2 RNA may also be prevented by the inability of pol III to terminate transcription at the U2 gene termination signal¹⁵ or by inappropriate RNA capping¹⁶.

We prepared two derivatives of the U6syn/U2 hybrid, U6syn+10a/U2 and U6syn+10b/U2, each having an additional 10 bp of different sequence inserted between the USE and TATA box (Fig. 2a). Transfection of these genes resulted in accumulation of U2 RNA at a level comparable to that seen



(ref. 12) pre-cut with *Bam*HI and *Cla*I. Mutated constructs U6syn+10a/U2 and U6syn+10b/U2 were generated from U6syn/U2 by replacement of the *Xho*I-*Nco*I fragment with a longer linker having the sequence CTCGAG-TAAAAATAAGAAAGATTCCATGG or CTCGAGTTATGGAATTTCTTCTACCATGG. Expression was tested in protoplasts of *N. plumbaginifolia*^{26,27}. Subsaturation amounts (10 μ g) of plasmids were used. RNA was prepared 24 h after transfection and used (3 μ g) for RNase A/T₁ mapping^{26,27}. The U6 and U2 gene-specific antisense RNA probes, of 440 and 510 nucleotides, were synthesized with SP6 polymerase using [α -³²P]GTP and pU6-26 (linearized with *Bst*XI) or pGEM2.U2.2 (linearized with *Hind*III)¹⁸ as templates. Protected fragments were quantitated^{26,27} and activity normalized for transfection efficiency as measured by expression of the reference gene.

FIG. 2 Conversion of the U6 gene promoter into a pol II-specific promoter. a, Schematic representation of the U2syn gene and of the hybrid U6syn/U2 genes. The U2syn and U6syn gene sequences of the hybrid genes are shown as open and stippled boxes, respectively. The relative activities of the different genes are indicated (averages from three independent transfections). b, RNase A/T₁ mapping. Protoplasts were transfected with plasmid pU6-26 (containing reference U6 gene) alone (lane 3) or with pU6-26 plus the genes indicated above lanes 4-9. The upper and lower panels show mapping with U2 and U6 probes, respectively. Two different U2-specific probes, the wild-type U2 gene probe (lanes 4-9) and the U6syn/U2-specific probe (data not shown; these probes differ in sequences upstream of position +1) yielded identical protected fragments, indicating that initiation occurred at the A at position +1 and that transcripts were properly terminated. M, size markers (in nucleotides). The 93-97-nucleotide bands (*) result from the partial digestion of the U6 RNA-specific hybrid by RNase A at the AU-rich 3' end. c, Effect of α -amanitin on expression of the gene U6syn+10a/U2. Protoplasts were cotransfected with pU6syn+10a/U2 and pU6syn and incubated in the presence or absence of 50 μ g ml⁻¹ α -amanitin. Mapping with U2 and U6 probes is in lanes 3-5 and 6-8, respectively. METHODS. The gene U2syn is a derivative of U2.SP, the *Arabidopsis* U2 RNA gene that contains a synthetic 98-bp promoter; pU2syn was constructed similarly to pU2.SP (ref. 12). The U2syn gene differs from U2.SP by four nucleotide substitutions in the -22 to -8 region that create *Nde*I and *Aat*II sites (Fig. 3b). The activities of the U2.SP, U2syn and the authentic U2 gene are comparable¹². The plasmid pU6syn/U2 was constructed similarly to pU2syn (ref. 12). The U6syn promoter sequence (position -70 to -1; see Fig. 3) was assembled from five oligonucleotides and inserted into pU2.2/*Cla*I

with the gene U2syn, in which synthesis of U2 RNA is driven by a synthetic U2 gene promoter (Fig. 2b). Transcription of the gene U6syn + 10a/U2 was inhibited by α -amanitin as expected¹⁷ for a gene transcribed by pol II; transcription of the cotransfected control U6 gene was α -amanitin resistant (Fig. 2c). It is possible, therefore, to change the pol III-specific U6 gene promoter into an efficient pol II-specific promoter by inserting 10 bp of DNA between the USE and the TATA-like box. It should be noted that each of the inserted sequences differs substantially from the sequences present in this region in the authentic U2 genes¹⁸.

To study the pol II to pol III promoter conversion we have constructed a hybrid gene, U2syn/U6, in which the 98-bp synthetic promoter¹² of the gene U2syn is fused with the coding and terminator regions of the gene U6syn. We also made a U2syn/U6 derivative, U2syn-10/U6, in which the distance between the USE and TATAAATA was shortened by 10 bp to conform with the spacing in pol III-specific promoters (Fig. 3a). Neither of the two genes was active in synthesis of U6 RNA (Fig. 3c). We have noted that the distance between the TATA-like sequence and the transcription start site is 1-4 bp shorter in U6 genes as compared with the pol II-specific genes (Fig. 1) and, in particular, 2 bp shorter than in the genes

U2syn/U6 or U2syn-10/U6 (Fig. 3b). We have therefore prepared three variants of each of these two genes, in which the TATA to +1 distance is shortened by 2, 3 or 4 bp (Fig. 3a and b). None of the constructs of the U2syn/U6 series (U2syn(-2, -3 or -4)/U6) was active but two of the constructs containing, in addition, a 10-bp deletion between the USE and TATA, U2syn-10(-2)/U6 and U2syn-10(-3)/U6, yielded U6 RNA (Fig. 3c). The U6 RNA synthesis from U2syn-10(-2)/U6 (Fig. 3d) and U2syn-10(-3)/U6 (data not shown) was α -amanitin-resistant as expected for genes transcribed by pol III. Hence, it is also possible to change a pol II promoter into a pol III promoter by manipulating the spacing between the USE and TATA elements, provided that the distance between the TATA-like sequence and the initiation site conforms to the U6 gene consensus.

It is unlikely that the 1-4-bp difference in the TATA to +1 distance found between pol II and pol III U snRNA genes is important for polymerase selection. The gene U6syn + 10/U2, for example, has a pol III-like TATA to +1 spacing, but is very efficiently transcribed by pol II (Fig. 2). It is more probable that pol II and pol III themselves differ slightly in the properties responsible for specifying the TATA to +1 distance. It has been demonstrated that factors other than the TATA box-binding

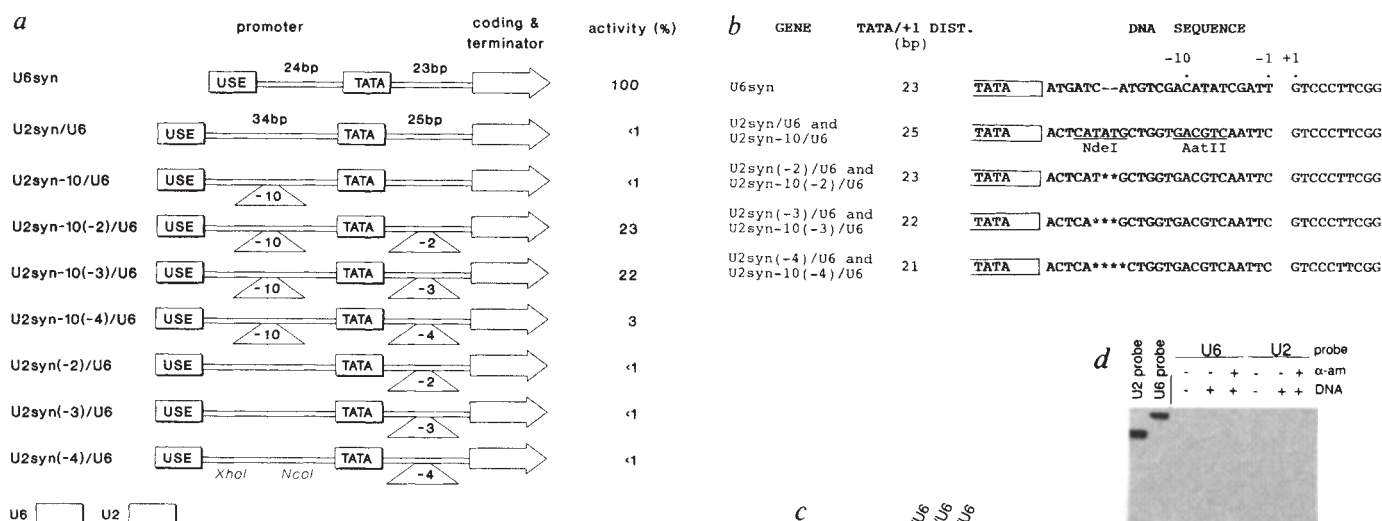


FIG. 3 Conversion of the U2 gene promoter into a pol III-specific promoter. **a**, Schematic representation of different genes and their activities (for more details see text and Fig. 2a). **b**, sequences of the -25 to +10 regions of the genes in (a). *, Nucleotides deleted in some mutated constructs. The NdeI and AatII sites in U2syn/U6 are underlined. The distance between the TATA element (boxed) and position +1 is indicated. **c**, RNase A/T₁ mapping with U6 and U2 probes. Plasmids used for cotransfection with pU2.2, which contains the reference U2 gene¹², are indicated at the top. Mapping of U6 RNAs transcribed from these plasmids was carried out with probe U6-26 (lanes 2-10), which is complementary to the upstream nucleotides in genes U6-26 and U6syn but not in genes of the U2syn/U6 series and with the probe specific for the gene U2syn/U6 (data not shown). The protected fragments were of identical lengths, indicating that initiation occurred at the G at position +1 and that transcripts were properly terminated. **d**, Effect of α -amanitin. Protoplasts were cotransfected with pU2syn-10(-2)/U6 and pU6syn. Mapping with U6 and U2 probes, lanes 3-5 and 6-8 respectively. METHODS. The 188-bp U6syn sequence, assembled from 10 overlapping oligonucleotides, is described in detail elsewhere¹³. The 70-bp upstream region of U6syn contains the USE and TTTATATA elements at the conserved positions. The remaining upstream sequence is mostly unrelated to that of the authentic U6 genes and contains several restriction sites. The coding region is identical to the wild-type sequence and a stretch of T residues is included downstream as a terminator. The U6syn was transcribed with 60-65% efficiency compared with the authentic U6 RNA genes. The parent U2syn/U6 hybrid gene, including 98 bp of U2syn promoter (see Fig. 2) fused with the coding and terminator regions of U6syn, was prepared by annealing

and ligation²⁶ of 11 oligonucleotides. Synthetic U6syn and U2syn/U6 fragments were inserted into plasmid pTZ19R pre-cut with *Bam*HI and *Sph*II. The variants U2syn(-2, -3 or -4)/U6 were obtained by cleavage of pU2syn/U6 with *Nde*I, followed by limited digestion with mung bean nuclease and religation. The variants having a 10-bp deletion in the USE/TATA region were obtained by replacement of the 29-bp *Xho*I-*Nco*I fragment¹² with the shorter linker CTCGAGTAAATCCATGG.

protein (possibly RNA polymerase itself) 'measure' this distance during transcription by pol II^{19,20}. The inability of pol III to initiate transcription from the U2syn-10/U6 and U2syn-10(-4)/U6 genes may be due to the absence of a pyrimidine-purine dinucleotide located at the correct (22-23 bp) distance from the TATA box (Fig. 3b). Transcription of all plant U snRNA genes starts at a purine residue preceded by a pyrimidine^{15,18,21,22}. Transcription of the U6 gene of *Xenopus* has similar requirements⁶.

Our experiments do not formally eliminate the possibility that intragenic sequences also contribute to RNA polymerase selection. But the identification of two other pol III-specific gene classes in *Arabidopsis* having USE/TATA spacing similar to that of U6 genes (T. Kiss, Ch. Marshallsay and W. F., manuscript in preparation) and the demonstration that the intragenic A box-like sequence found in *Arabidopsis* U6 genes is not required for transcription¹³ make this possibility unlikely.

The mechanism by which differences in spacing between two promoter elements in plant U snRNA genes determine the choice of RNA polymerase is not known. The mode of interaction between the proteins that bind the USE and TATA may depend on the spacing between them, giving rise to two alternative surfaces recognized by the appropriate polymerase complex or by an additional protein acting in between. Alternatively, distinct TATA or USE factors may interact with pol II and pol III promoters, specificity being governed by the proximity of the protein bound at the neighbouring site.

The structure of U snRNA gene promoters and the factors that define polymerase specificity are different in plant and vertebrate cells. Class II and class III promoters in vertebrates contain two common upstream elements, DSE and PSE, which are sufficient for transcription of the U2 gene by pol II (reviewed in refs 23, 24). The U6 genes contain an additional element, an AT-rich TATA-like box that confers pol III specificity^{6,9}. In plants, both classes of U snRNA genes contain essential -30 TATA-like elements and it will be of interest to determine whether the same or different TATA factors participate in pol II- and pol III-specific transcription.

Our results support the idea that polymerases II and III evolved from a common ancestor²⁵, as did pol II and pol III U snRNA gene promoters⁶, and that one mechanism of promoter diversification in eukaryotes involved changes in the spacing between promoter regulatory elements. □

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